ESTONIAN UNIVERSITY OF LIFE SCIENCES
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PREVALENCE AND AGE STRUCTURE OF AFRICAN SWINE FEVER SURVIVOR ANIMALS IN THE WILD BOAR POPULATION DURING THE EPIDEMIC IN ESTONIA

SIGADE AAFRIKA KATKU ÜLEELNAUD METSSIGADE LEVIMUS JA VANUSELINE STRUKTUUR POPULATSIOONIS EPIDEEMIA AJAL EESTIS

Final Thesis
Curriculum in Veterinary Medicine

Supervisor: Arvo Viltrop,
Chair Professor of Veterinary Bio- and Population Medicine

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African swine fever (ASF) is a highly lethal disease of suids. It was introduced into Georgia in 2007, and since then it has developed into an unprecedented and unresolved epidemic in Eastern Europe and Asia. The aim of this study was to describe the dynamics of the ASF epidemic in the wild boar population in Estonia, with emphasis on seropositive animals. This was achieved by analysing the surveillance data collected by the Estonian Veterinary and Food Board. The dynamics of seroprevalence were compared in different age classes. At the start of the epidemic, the average seroprevalence was higher in young wild boar, while in later phases, it was higher in older age classes. This was found to be statistically significant in most cases. Factors that potentially have an effect on these dynamics were identified by performing statistical analysis. The hypothesis was that the increased seroprevalence among young wild boar is related to the presence of virus-positive animals in the region. There was a statistically significant positive correlation between the number of virus-positive animals and young seropositive animals. The hypothesis was confirmed with univariable analysis. The duration of the ASF epidemic in Estonia was estimated by determining the time period of observation of virus-positive and seropositive animals in different age classes on a county level. It may be suggested that the duration of the ASF epidemic in Estonia was at maximum 18 calendar quarters (approximately 54 months), starting from the time of entrance of the infection into the territory of a county. After the 18th calendar quarter, no virus-positive animals or young seropositive animals were detected in any county that would indicate the circulation of the virus in the population.

Keywords: seroprevalence, viroprevalence, antibody carrier, disease dynamics, epidemiology

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Märksõnad: serolevimus, viiruse levimus, antikehakandja, haiguse dünaamika, epidemioloogia			

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LIST OF ABBREVIATIONS AND USED KEYS

ADNS – Animal Disease Notification System

ASF – African swine fever

ASFV – African swine fever virus

DPE – days post-exposure

DPI – days post-inoculation

EFSA – European Food Safety Authority

ELISA – enzyme-linked immunosorbent assay

EURL – European Union Reference Laboratory

HAT – Haemadsorption test

N (sero) - number of serologically tested wild boar in an age class in a calendar quarter in a county

N (viro) – number of PCR-tested wild boar in an age class in a calendar quarter in a county

P(sero) – seroprevalence

P (viro) – viroprevalence

PCR – polymerase chain reaction

PR – prevalence ratio

$P_{<1y}$ – average seroprevalence of wild boar less than one year of age in a year in a region

$P_{1\text{-}2y}$ – average seroprevalence of wild boar 1-2 years of age in a year in a region

$P_{>2y}$ – average seroprevalence of wild boar more than two years of age in a year in a region

qPCR – quantitative polymerase chain reaction

Q1 – the first calendar quarter in a year

Q2 – the second calendar quarter in a year

Q3 – the third calendar quarter in a year

Q4 – the fourth calendar quarter in a year

r – Pearson correlation coefficient

S - number of seropositive wild boar in an age class in a calendar quarter in a county

V - number of PCR-positive wild boar in an age class in a calendar quarter in a county

<1y – wild boar less than one year of age

≥1y – wild boar one year of age or older

1-2y – wild boar 1-2 years of age

>2y – wild boar more than two years of age

<1y_1 – number of seropositive animals in wild boar less than one year of age

%_Viro_1 – viroprevalence

%_<1Y_1 – seroprevalence in wild boar less than one year of age

%_≥1Y_1 – seroprevalence in wild boar one year of age or older

INTRODUCTION

African swine fever (ASF) is a highly lethal viral disease of wild and domestic suids. It is notifiable to the World Organization of Animal Health (OIE, 2019). ASF is caused by the African swine fever virus (ASFV), a double-stranded DNA virus, the only member of the *Asfarviridae* family. The clinical presentation of this disease can range from acute haemorrhagic disease to subclinical infection (Reis *et al.*, 2007; Petrov *et al.*, 2018).

African swine fever is endemic in several sub-Saharan countries and Sardinia (Reis *et al.*, 2007; Petrov *et al.*, 2018). In 2007, ASF was introduced into Georgia and has since developed into an unprecedented and unresolved epidemic affecting Eastern Europe, Trans-Caucasus countries, the Russian Federation, and, recently, China and Southeast Asia (Petrov *et al.*, 2018; Chenais *et al.*, 2019; Eblé *et al.*, 2019; Sánchez-Cordón *et al.*, 2019).

Transmission is principally associated with movement of infected domestic pigs and wild boar as well as ingestion of contaminated pig products. ASFV has demonstrated a huge capacity of transboundary and transcontinental spread – the virus is able to survive for more than two years in frozen organs and chilled blood (Chenais *et al.*, 2019). It also has the ability to survive the process of putrefaction and persist in soil surrounding carcasses for several months. Long-distance spread is likely due to anthropogenic factors, such as transport of contaminated meat (Chenais *et al.*, 2019).

There is an ongoing debate about the possible role of subclinical ASFV carriers in maintaining and perpetuating spread of ASF (Ståhl *et al.*, 2019). The existence of such carriers is controversial and has yet to be proven definitively, however, some sources refer to them as an established fact (Schlafer *et al.*, 1984a; de Carvalho Ferreira *et al.*, 2012). A subclinical carrier of ASFV could be defined as an individual that has survived ASF, is apparently healthy, and a persistent or chronic infection has resulted in constant or intermittent shedding of ASFV (Petrov *et al.*, 2018). In an industrial setting, this may not necessarily play an important role, as there are radical disease control measures in place such as stamping out. In the wild boar population, however, subclinical carriers may maintain the

virus in the population and continue spreading it to naïve animals for months or years. Should these concerns prove true, ASFV carriers would be a risk factor that could not be easily eliminated. This can be especially relevant in areas where a lower case-fatality and higher survival rates of ASF are reported (Petrov *et al.*, 2018).

The literature review section of this thesis provides an analysis of the current data of the prevalence and epidemiological role of wild boar that have survived ASF (i.e. are seropositive), the age structure and age dynamics of such animals, during different phases of the epizootic. The effect of passively derived anti-ASFV antibodies on the clinical course of ASF in neonatal and young pigs is investigated as well. In addition, the general characteristics of ASF as a disease in terms of epidemiology, clinical signs, virological and serological responses, as well as current control strategies are reviewed.

The aim of this study was to determine the dynamics of the ASF epidemic in the wild boar population of Estonia in 2014-2019. Particular emphasis was put on wild boar that survive ASF (i.e. are seropositive). Factors that potentially have an affect seroprevalence in different age classes were investigated. The hypothesis was that increased seroprevalence in young wild boar is related to the presence of ASFV-positive animals in a region. In addition, the duration of the ASF epidemic on the county level in Estonia was estimated. To do this, the official African swine fever surveillance data collected by the Estonian Veterinary and Food Board was consolidated into a county-level dataset in Microsoft Excel. The Estonian counties were divided into three regions, South-east, North-east and West, depending on when the incursion of ASF occurred. Then, analysis of seroprevalence in different age classes as well as viroprevalence was done by illustration in graphs and performing statistical analysis on a regional level. The duration of the ASF epidemic in Estonia was estimated by determining the duration of observation of ASFV-positive animals and seropositive animals on a county level and performing statistical analysis. The dynamics of seroprevalence and viroprevalence were also evaluated on a country level.

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I would also like to thank my family for being there through this journey, for encouragement and support always.

AIMS OF THE STUDY

The general aim of this study was to describe the dynamics of the African swine fever epidemic among wild boar in Estonia in the time period of 2014-2019, with special emphasis on surviving (seropositive) animals. The specific goals were:

- to compare the dynamics of the seroprevalence of African swine fever in different age classes of wild boar;
- to identify factors that have a potential effect on the seroprevalence in the age classes. The hypothesis was that the presence and increased seroprevalence among young wild boar in a region is related to the presence of ASFV-positive animals in that region;
- to estimate the duration of the African swine fever epidemic on the county level;
- to estimate the duration of observations of seropositive wild boar in different age classes;
- to estimate the duration of observations of ASFV-positive wild boar.

1. LITERATURE REVIEW

1.1. Epidemiology of African swine fever

There are 23 known genotypes of ASFV. Genotype I spread to Spain and Portugal in 1957 and 1960 and is currently in Sardinia, whereas Genotype II was introduced into Georgia in 2007 and initiated the current pandemic (Figure 1) (Nurmoja *et al.*, 2017a; Sánchez-Cordón *et al.*, 2018; Chenais *et al.*, 2019).

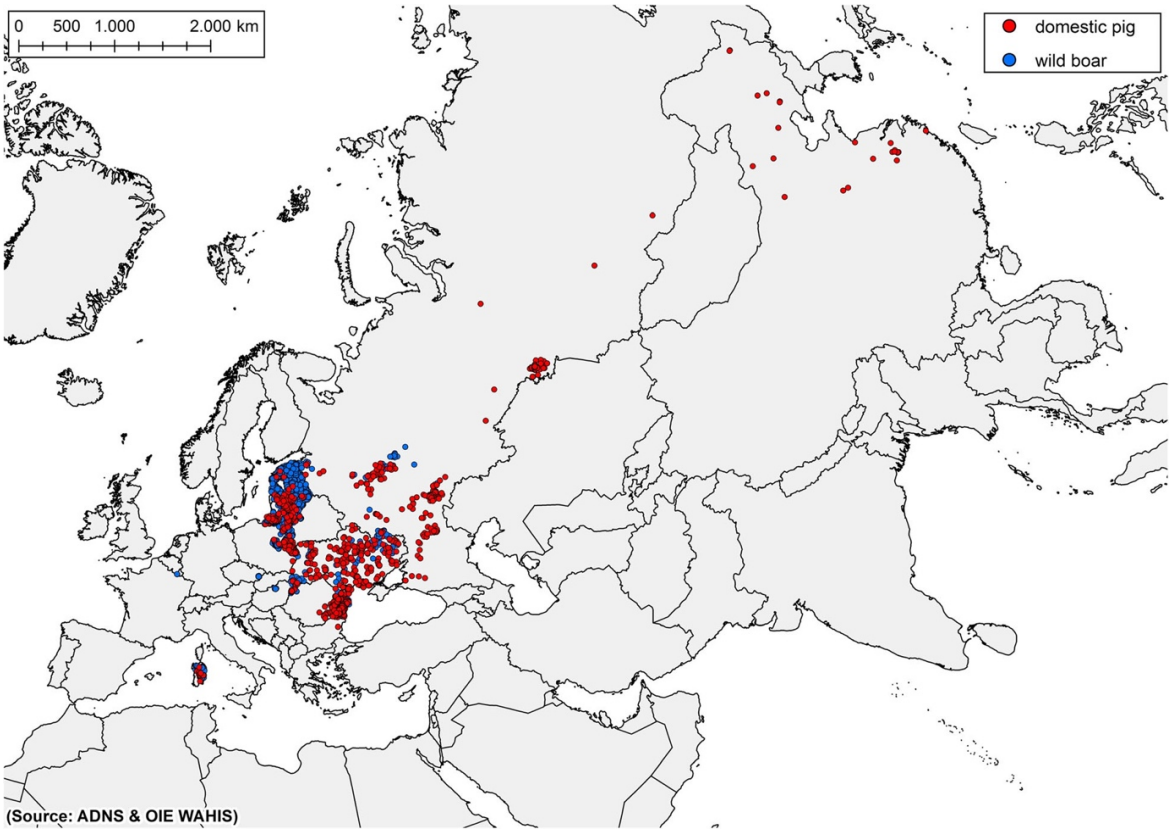

Figure 1. Notifications of African swine fever cases in wild boar (blue colour) and outbreaks in domestic pigs (red colour). Data from the Animal Disease Notification System (ADNS) from January 2017 until September 2018 (Chenais *et al.*, 2019).

Originally, the main components of ASFV spread were soft ticks of *Ornithodoros* species, warthogs (*Phacochoerus africanuus*), and bushpigs (*Potamochoerus larvatus*), which are

the natural reservoirs of ASFV in Africa (Chenais *et al.*, 2018). The three original epidemiologic cycles of ASF are:

1. Sylvatic cycle. This cycle is the origin of ASF as a disease. The main reservoirs and components of ASFV spread in Africa are soft ticks of *Ornithodoros* species, warthogs (*Phacochoerus africanuus*), and bushpigs (*Potamochoerus larvatus*).
2. Tick-pig cycle. ASFV circulates between *Ornithodoros* species of ticks and domestic pigs (*Sus scrofa domesticus*), with the ticks serving as a reservoir. This cycle perpetuated the Iberian Peninsula epizootic.
3. Domestic cycle. ASFV circulates among domestic pigs and transmission occurs mainly by ingestion of contaminated pig products. This cycle does not involve the natural reservoirs (Chenais *et al.*, 2018).

The three aforementioned epidemiologic cycles are independent, and they can overlap (Figure 2) (Chenais *et al.*, 2018).

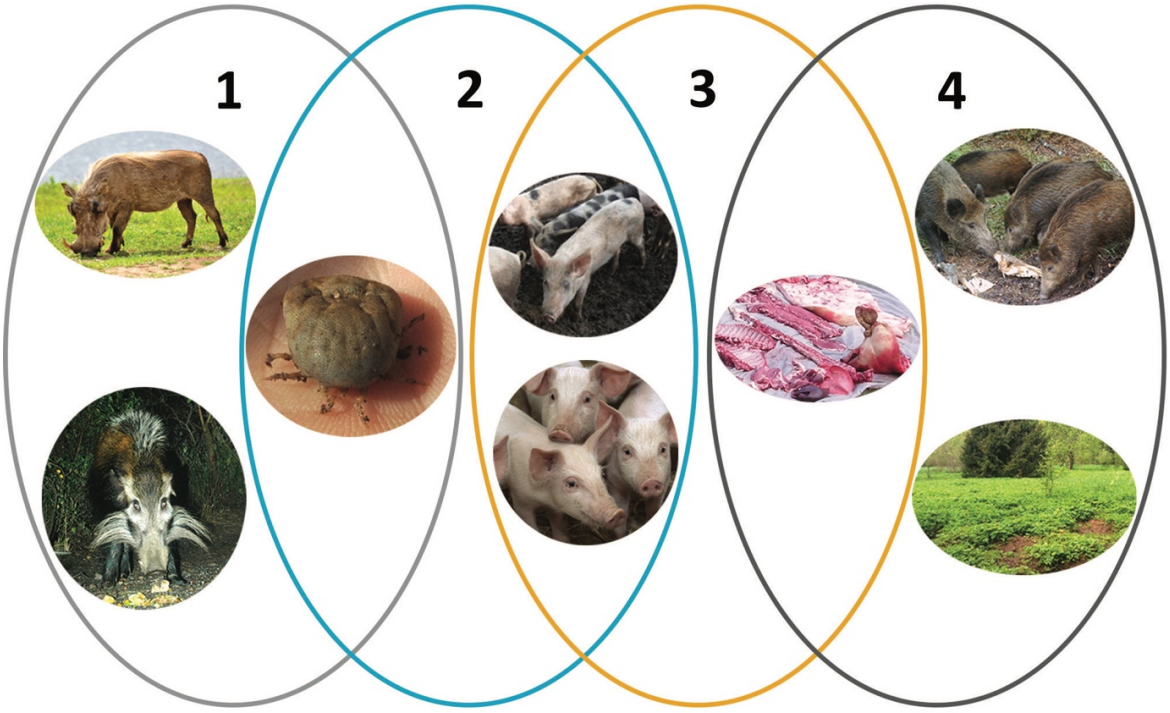

Figure 2. The four defined epidemiological cycles of ASFV: 1) sylvatic cycle - the common warthog (*Phacocoerus africanuus*), bushpig (*Potamochoerus larvatus*), and soft ticks (*Ornithodoros* species); 2) tick-pig cycle - soft ticks and domestic pigs (*Sus scrofa domesticus*); 3) domestic cycle - domestic pigs and pig products (meat, lard, bones, bone marrow, skin); 4) wild boar-habitat cycle - wild boar (*Sus scrofa*), domestic pig- and wild boar- derived products, carcasses, and the habitat (Chenais *et al.,* 2018).

Taking the events of the current epidemic into account, it has been suggested to define a fourth epidemiologic cycle – the wild boar-habitat cycle (Figure 2). This cycle was described based on data collected from the common reporting framework in the European Union (EU). Here, the ASFV reservoir is the environment, and the virus circulates among the wild boar (*Sus scrofa*) population via direct contact between infected and susceptible animals and by indirect transmission via contaminated carcasses. ASFV persistence in the environment is favoured by a cold, moist climate (Chenais *et al.*, 2018).

1.2. Control and prevention of African swine fever

In the wild boar population, effective control of ASF depends on the current disease status of a particular area. The main principles, however, are similar in all scenarios. Passive surveillance systems for early detection of ASF in wild boar are of paramount importance in affected areas and those that are as yet ASF-free (EFSA *et al.*, 2018). Attention should be paid to the ecology of wild boar, current hunting practices and biosecurity. In affected areas, wild boar depopulation is carried out by efficient hunting, and the boar that are found dead are removed from the environment promptly and under conditions of high biosecurity. Efforts are also made to stabilise wild boar populations by implementing feeding bans and improving crop protection (EFSA, 2018; Jurado *et al.*, 2018).

In areas where ASF has been present in the wild boar population for more than one year, in addition to measures described above, the age profile of seropositive animals should be determined (EFSA, 2018).

In the domestic pig sector, control of ASF includes surveillance, epidemiological investigation, tracing pigs and pig products, strict quarantine and biosecurity measures, as well as animal movement control in domestic pig holdings. In case an outbreak occurs, infected holdings are stamped out (Jurado *et al.*, 2018).

Biosecurity in a pig farm consists of two components, neither of which can be effective without the other – biosecurity hardware and software. Hardware entails buildings, fences,

equipment, roads. Software is seen as the biosecurity-oriented mindset and education of personnel (Jurado *et al.*, 2018; Chenais *et al.*, 2019). Social factors play a much more important role in ASF spread than previously acknowledged. Thus, personnel and smallholder education about biosecurity measures to prevent ASF is of paramount importance. In a backyard setting, swill feeding presents one of the most important risk factors (Jurado *et al.*, 2018; Lamberga *et al.*, 2018; Chenais *et al.*, 2019).

1.3. Biological characterization of African swine fever virus Genotype II

Clinical courses of ASF are variable, ranging from peracute (manifesting as sudden death with no gross lesions) to chronic and subclinical infection, with variable incubation periods, clinical signs, and case fatality (Reis *et al.*, 2007; Sánchez-Cordón *et al.*, 2018; Chenais *et al.*, 2019).

In order to precisely evaluate clinical signs, horizontal transmission, the humoral and virological response, an experimental infection was conducted at the European Union Reference Laboratory (EURL) by Gallardo *et al.* (2017), whereby eight domestic pigs were infected with the Lithuania 2014 (LT14/1490) field isolate. ASFV LT14/1490 is a Genotype II virus, and thus is representative of the ongoing epizootic. Ten naïve pigs were then placed in contact with the inoculated pigs. Clinical signs were monitored and scored daily, and blood samples were collected twice weekly. After succumbing to disease or slaughter, necropsy was performed on all animals, and 20 types of tissues were collected from each pig to extract viral DNA.

Both the infected and in-contact animals generally displayed similar clinical signs and died or were slaughtered 3-4 days after they became apparent (Gallardo *et al.*, 2017). Seven of eight inoculated animals succumbed to the disease or were slaughtered 7-9 DPI (days post-inoculation) (Gallardo *et al.*, 2017). One inoculated pig showed a delayed course of disease, similar to that seen in the in-contact animals. The commonly observed clinical signs included fever, followed by hypothermia shortly before death, ecchymoses and cyanosis of various areas on the skin, ocular discharge and bloody diarrhoea. Pathological lesions found at

necropsy included oedematous and haemorrhagic lymph nodes, hyperaemic splenomegaly, hydropericardium, hepatic congestion, as well as petechiae in the renal cortex and medulla. ASFV genome was detectable in all sampled organs and tissues (Gallardo *et al.*, 2017).

Similar results in terms of observed clinical signs and pathomorphological lesions were obtained by Pietschmann *et al.* (2015) in an experimental study using another Genotype II isolate ("Armenia08"), where both domestic pigs and wild boar were inoculated. Petrov *et al.* (2018) also reported similar results using a Genotype I isolate, though the onset of clinical signs was more variable, and some animals showed a sporadic re-emergence of clinical signs after periods of apparent convalescence. This phenomenon was also reported by de Carvalho Ferreira *et al.* (2012), using several different ASFV isolates.

In seven inoculated pigs, ASFV genome was first detected in blood at 3.75 ± 1.4 DPI. Maximum titres were seen at 6 DPI and at 14 DPE (days post-exposure) in inoculated and in-contact animals, respectively (Gallardo *et al.*, 2017). One in-contact pig remained asymptomatic throughout the experiment and showed intermittent, weak viremia at 17, 34 and 38 DPE (Figure 3). ASFV genome was detected in several tissues at necropsy, but the virus could not be isolated in this animal, and no antibody response was observed (Gallardo *et al.*, 2017).

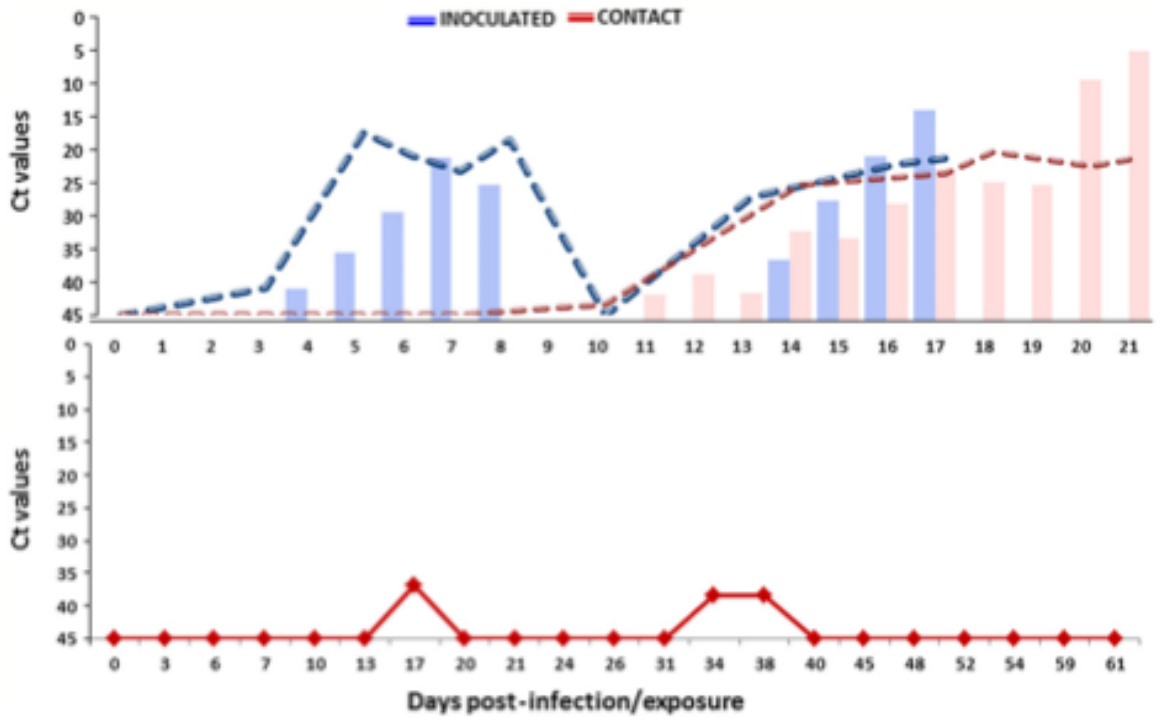

Figure 3. Top – viremia determined using real-time PCR in the groups of inoculated (blue interrupted line) and in-contact animals (red interrupted line), overlapped with the clinical score (blue and red bars, respectively); bottom – viremia determined by real-time PCR in the survivor animal (Gallardo *et al.*, 2017).

In total, 94.5% mortality was seen in this experiment. In the inoculated animals, the incubation period was 4-5 days, and a delay of 12-14 days on average was seen in the in-contact pigs, which developed severe disease and became moribound at 18-22 DPE (Gallardo *et al.*, 2017). ASFV genome was detectable by PCR (polymerase chain reaction) before the appearance of clinical signs. These results are likely representative of disease dynamics in domestic pigs in an industrial setting (Gallardo *et al.*, 2017).

A different experimental infection was performed by Reis *et al.* (2007) to determine the role of antibodies in the pathogenesis and diagnosis of ASF. Here, 15 pigs were inoculated with the ASFV/NH/P68 isolate, which is non-fatal and non-haemadosrbing. The total antibody and IgG responses were evaluated longitudinally against 12 viral proteins, which were divided into three groups based on the observed response, ranging from a strong antibody response to a weak response. Furthermore, the viral proteins that elicited a strong humoral response were also tested by ELISA (enzyme-linked immunosorbent assay) in order to determine if it was possible to identify recently infected animals by their IgM responses.

The IgM response was detectable at 7 DPI and began to decrease at 14 DPI (Reis *et al.,* 2007). Animals could be detected as infected using the K205R protein. Antibodies could be detected in all animals at 11 DPI. It was found that animals that developed lesions showed a significantly higher antibody response to the proteins NP419/DNA ligase, CP312R, B646/p73, K196 thymidine kinase and K205R (Reis *et al.,* 2007). No significantly different response was seen in asymptomatic and chronically infected animals. A higher response was seen in asymptomatic animals towards the protein A104/histone-like, but this finding was not statistically significant (Reis *et al.,* 2007).

It was concluded by Reis *et al.* (2007) that ASFV proteins E183/p54, K205R, A104/histone-like, and B602L are potentially diagnostic serological antigens, as all of them showed 100% sensitivity at 21 DPI. Additionally, K205R could be used to detect early IgM responses. Moreover, determining the IgM:IgG ratio would be useful in distinguishing recently infected and chronically infected individuals, as IgG levels increased significantly on 14 DPI, followed by a marked decrease in IgM.

1.4. The role of survivor (antibody-positive) animals in the epidemiology of African swine fever

One of the more controversial debates on the subject of ASF as a disease is the existence of subclinical carriers of ASFV, which could then continue transmitting the virus to naïve animals for extended periods of time (Ståhl *et al.*, 2019). This has arguably been the case since the disease was first defined. Moreover, some articles actually refer to subclinical carriers as though their existence had been proven definitively and they had a measurable impact on the spread of ASF (Schlafer *et al.*, 1984a; Allaway *et al.*, 1995; de Carvalho Ferreira *et al.*, 2012; Eblé *et al.*, 2019).

Most evidence indicates that animals that have recovered from ASF are able to eliminate the virus completely and do not transmit it to naïve animals, even if the naïve animals have been in close contact to the "survivors" (antibody-positive animals) for an extended period of time. (Penrith *et al.*, 2004, Pietschmann *et al.*, 2015; Petrov *et al.*, 2018). One study claims to have proven the contrary, however. An experimental study was performed by Eblé *et al.*

(2019), to determine whether ASFV carriers exist and whether they transmit the virus to naïve animals. In this experiment, ASFV transmission was indeed demonstrated.

Petrov *et al.* (2018) used the same isolate as Eblé *et al.* (2019), ASFV'86, yet they obtained contradicting results (see Table 1). In the study by Petrov *et al.* (2018), 30 domestic pigs were inoculated, allowed to recover, remixed and then co-mingled with six naïve sentinel pigs at 99 DPI. At this point, 19 inoculated pigs were still alive after surviving the acute phase of disease. The highest genome loads were detected by PCR at 10 DPI and started to decrease at 29 DPI (Petrov *et al.*, 2018). The first negative results were obtained at 42 DPI, also, all oral swab samples tested negative by PCR at this point. At 91 DPI, 52% of pigs still had positive qPCR results, but the genome loads were decreased. At 63 DPI, however, all surviving animals tested negative by HAT (haemadsorption test) (Petrov *et al.*, 2018). Furthermore, during two months of cohabitation with inoculated pigs, no clinical signs or pathomorphological changes were observed in the sentinel animals, and qPCR (quantitative PCR) and HAT were negative in all sample matrices. The inoculated pigs remained antibody positive until the termination of the trial at 164/165 DPI (Petrov *et al.*, 2018).

The study by Petrov *et al.*, (2018) shows that antibody production and clinical outcome of ASF are not well correlated. Animals that recovered from disease were capable of fully eliminating ASFV, yet it required more than two months (viable virus was not detected starting from 63 DPI). The results of this study suggest that ASF survivors may not play a significant role as ASFV carriers, at least in a setting that lacks the biological vectors – ticks of *Ornithodoros* species.

In the study by Eblé *et al.* (2019), the same ASFV isolate was used to inoculate six domestic pigs. At 28 DPI, naïve sentinel animals were introduced to the infected ones and removed at 41 DPI (C1). At 42 DPI, new sentinel animals were introduced (C2). Clinical signs were observed in two C2 pigs in the period 42-55 DPI. In these pigs, serum samples and oropharyngeal fluid samples tested positive for ASFV by qPCR and virus isolation. In this study (Eblé *et al.* 2019), ASFV by direct contact was demonstrated, and these results contradict those of the study by Petrov *et al.* (2018).

It should be noted that even though ASFV transmission by direct contact was observed in the study by Eblé *et al.* (2019), and these results contradicted those of Petrov *et al.* (2018),

naïve sentinel animals were co-mingled with inoculated animals at different stages of infection in the respective studies. Petrov *et al.* (2018) introduced sentinel animals to recovered animals at 99 DPI, and the animals that demonstrated clinical signs of ASF in the study by Eblé *et al.* (2019) were co-mingled at 42 DPI. It can be argued that at 42 DPI, the infected animals could be undergoing a chronic phase of infection and may not be considered truly convalescent at this point, and thus may not be considered true asymptomatic carriers of ASFV.

Table 1. Comparison of the outcome of the studies by Petrov *et al.* (2018) and Eblé *et al.* (2019) in terms of whether ASFV transmission could be demonstrated from experimentally infected to naïve pigs

Study	ASFV isolate used	Time after inoculation at introduction of naïve pigs, DPI	ASFV transmission observed from inoculated to naïve animals
Petrov *et al.,* 2018	ASFV'86	99	No
Eblé *et al.,* 2019	ASFV'86	28 and 42	Yes

Notes:

- ASFV - African swine fever virus;
- DPI - days post-inoculation

The contradicting results of the two studies described above demonstrate the need for a universally accepted definition of an ASFV carrier animal. Petrov *et al.* (2018) argue that an ASFV carrier may be defined as an individual that has survived ASF and is apparently healthy and a persistent or chronic infection has resulted in constant or intermittent shedding of ASFV. Petrov *et al.* (2018) also point out the need to define and differentiate the terms "long-term persistence", "persistent infection" and "chronic infection" – this is a matter of further discussion and research.

1.5. The prevalence and age structure of seropositive and virus-positive pigs

A relevant aspect of determining the existence and role of asymptomatic ASFV carriers is determining the prevalence and age structure of animals that are ASFV antibody-positive and are currently not displaying clinical signs of ASF. This would be an important tool in understanding the epidemiology of the disease and aid in the design of control strategies and

their implementation (Kouokam *et al.*, 2013). There are few studies and a significant lack of data on this subject, especially in the context of the current ASF epizootic in the EU, the Russian Federation and Asian countries. A study was carried out by conducting a farmer questionnaire and serological survey in two districts in Malawi by Allaway *et al.* (1995). It was found that the prevalence of anti-ASFV antibodies was 12.4% (55 of 445 pigs sampled in 35 villages). The highest proportion of seropositive pigs was in the age group of 18-23 months, but there was a number of seropositive animals in all age groups[1].

In the context of the ongoing ASF epizootic in Estonia, Nurmoja *et al.* (2017b) found that there was a higher probability to detect a seropositive or ASFV-positive animal in the juvenile (less than one year of age) than in the adult (more than one year of age) wild boar age class. This finding was statistically significant.

Another study was carried out by Walczak *et al.* (2020) with a focus on young wild boar (one year of age or less) in order to elucidate possible routes of obtaining seropositive results in samples collected in the time period of 2017-2018 in Poland. Active prevalence was determined to be 1.5%. In addition, 70% of the positive samples were positive for anti-ASFV antibodies and negative for ASFV DNA. In total, 292 samples were seropositive and PCR-negative, and 126 of these were obtained from wild boar aged one year or less.

Three hypotheses were considered by Walczak *et al.* (2020) to explain the phenomenon of the high proportion of seropositive and PCR-negative animals: 1) samples may have been obtained from convalescent animals; 2) some animals may have been infected with an ASFV isolate of a low virulence; 3) vertical transmission of immunoglobulins may have occurred via colostrum from convalescent sows.

In the wild boar population in the Baltic States, among the wild boar that are found dead, the proportion of PCR-positive animals is much higher than animals that tested antibody-positive by ELISA (EFSA, 2018). The proportion of wild boar testing positive is always

[1] The percentage of seropositive animals in each age group is not available, as the bar graph of the original article cannot be seen due to a printing error. The actual numbers were not indicated in the main text of the original article.

higher in wild boar that are found dead than in hunted wild boar (Figure 4) (Nurmoja *et al*., 2017b; EFSA, 2018). Among hunted wild boar, the prevalence of animals that test positive by PCR or ELISA, remains below 5% (EFSA, 2018).

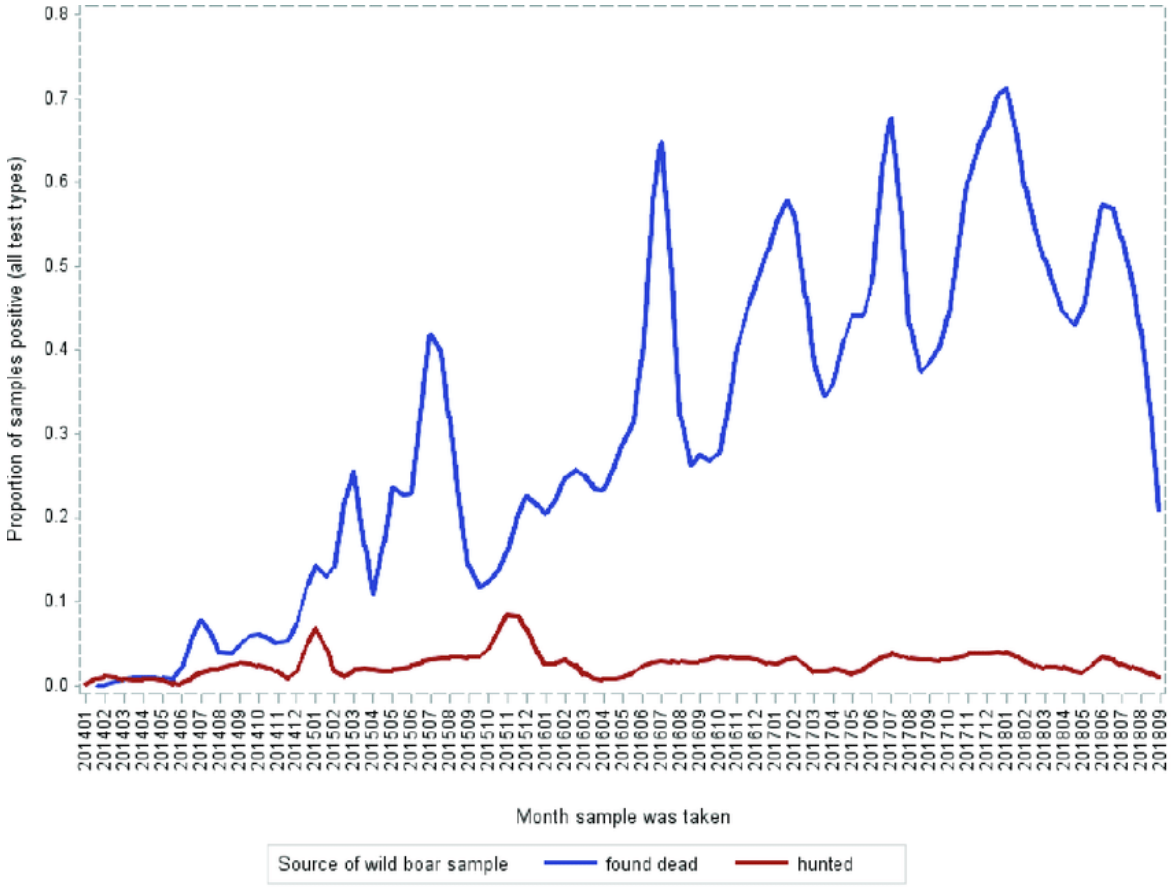

Figure 4. Proportion of positive samples in all tested samples (PCR, antibody-ELISA) in hunted wild boar (red colour) and those found dead (blue colour) in the Baltic Countries and Poland since the first introduction (2014 to 2018) (EFSA, 2018).

The seasonality of ASF notifications in wild boar was also investigated by EFSA (2018). It was found that there were peaks in wild boar found dead that test positive by PCR or ELISA during winter and summer, while a slight decline is seen in spring (Figure 5). A peak in the number of ASF notifications in domestic pigs is seen in the summer (Figure 6). In wild boar, this seasonal variability may possibly be explained by the increased survival of ASFV in lower temperatures, which could result in a longer "infectious period" for indirect transmission in winter via contaminated environment and carcasses. Another possible explanation could be the seasonality of the hunting season, which is a source of bias – there may be an increase in the number of ASF notifications but not occurrence (EFSA, 2018).

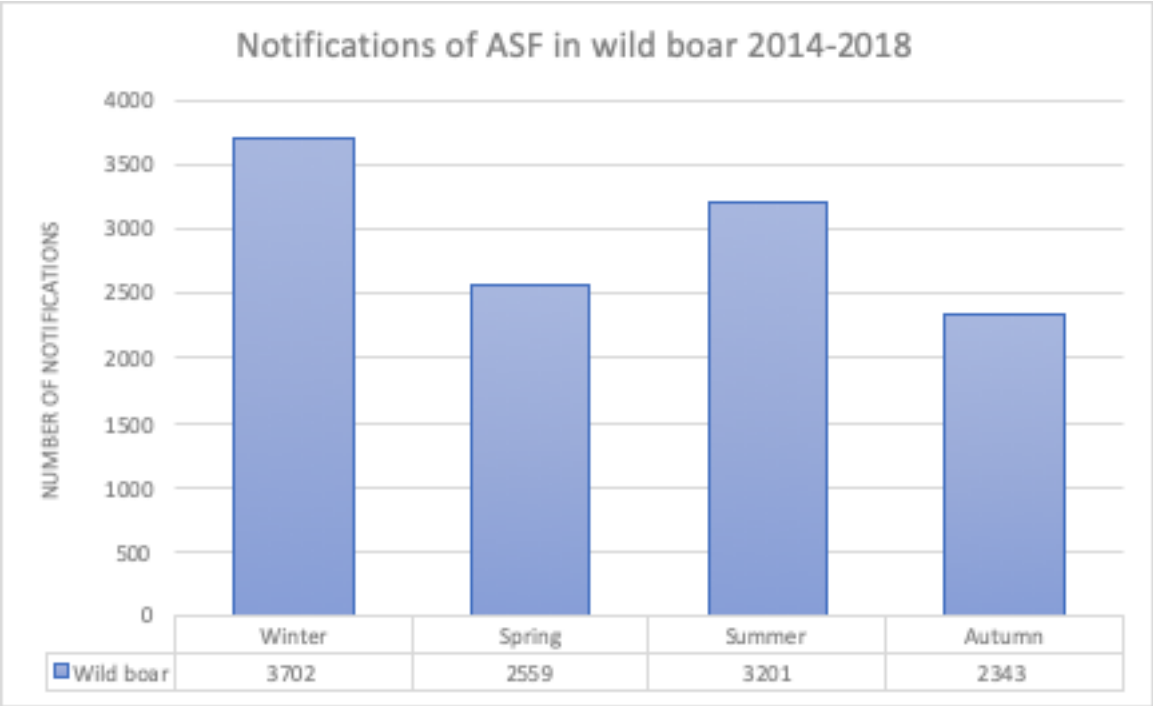

Figure 5. Number of ADNS (Animal Disease Notification System) notifications of ASF per season in wild boar from the Baltic countries and Poland from 2014 to 2018 (adapted from EFSA, 2018).

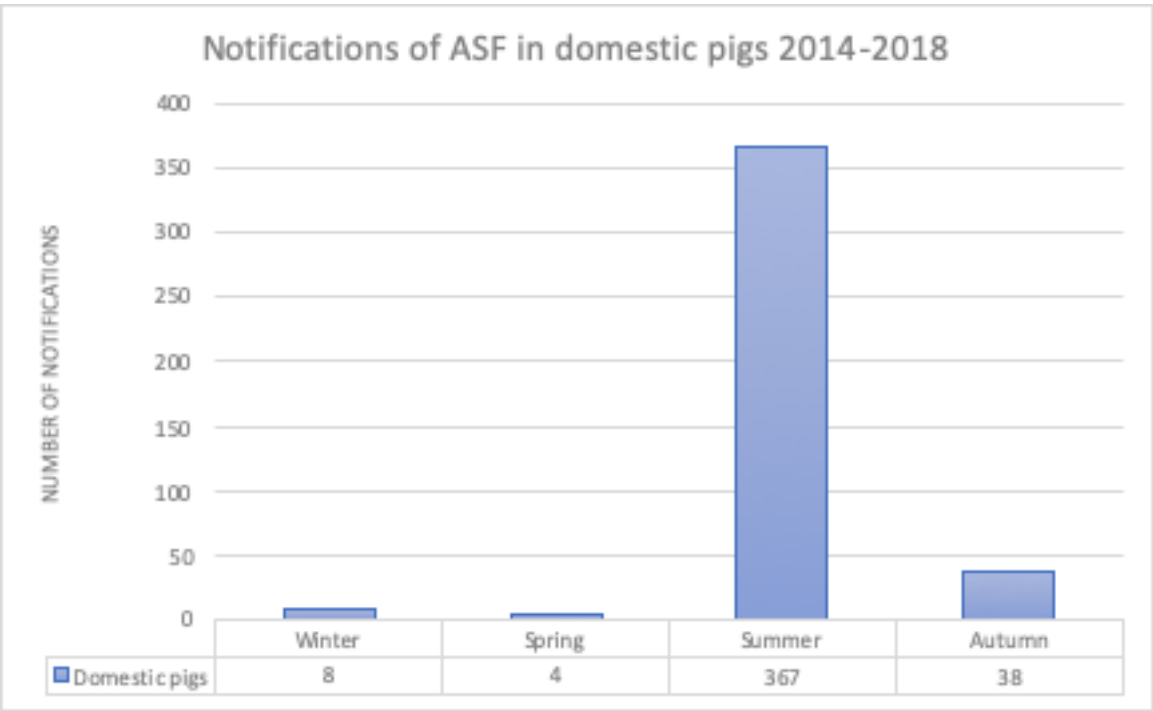

Figure 6. Number of ADNS (Animal Disease Notification System) ASF notifications of ASF per season in domestic pigs from the Baltic countries and Poland from 2014 to 2018 (adapted from EFSA, 2018).

The summer peak in the number of ASF notifications in domestic pigs may be explained by farming activities that show seasonal patterns. For example, the supply of freshly harvested litter in indoor farming systems usually happens during or shortly after summer, while in outdoor farming systems pigs are more likely allowed to roam outdoors during the summer (EFSA, 2018). Other farming activities indirectly related to pig farming may also contribute to this phenomenon, such as harvesting crops in the summer and preparing fields in the spring and autumn – this could increase the traffic of vehicles in and out of pig farms. Additionally, the feed composition may vary in accordance to the seasonal availability of ingredients (EFSA, 2018).

1.6. The role of passive immunity in the clinical course and survival rate of African swine fever in neonatal pigs

The subject of passive immunity is important in terms of its effect on the clinical course and duration of ASF in young pigs, as well as its effect on the survival rate, should they become infected with ASFV after birth. It has been reported from previous epizootics that ASF spread is faster in young pigs than in adults, but higher survival rates of young pigs in farms where there is high adult mortality have also been reported (Schlafer *et al.*, 1984b). Müller *et al.* (2005) estimated that the half-life of neutralising maternal antibodies in young wild boar against the pseudorabies virus was 21 days, and against the classical swine fever virus it was 11 days. The maternal antibodies against the classical swine fever virus persisted on a low level for several months. Studies of the half-life of neutralising antibodies in young pigs in the context of ASF are lacking at present.

Passive immunity may be acquired by the vertical transmission of IgG antibodies via two pathways – through the placenta and through the colostrum. However, in pigs, the placenta is of the epitheliochorial type and it is impermeable to immunoglobulins. Therefore, the only possible route of transferring immunoglobulins from dam to offspring in these animals is via colostrum (Walczak *et al.*, 2020). The primary factors of passive immunity are the antibody titres of the dam and the amount of colostrum ingested by the neonate (Müller *et al.*, 2005).

Müller *et al.*, (2005) argue that the long-term persistence of maternal antibodies on a low level may interfere with oral vaccination of young wild boar. Vaccination efforts against classical swine fever (less than one year of age) have proven to be unsatisfactory in the past. Should the development of a vaccine against African swine fever be successful in the future, this should be kept in mind when devising vaccination strategies.

Schlafer *et al.* (1984a) evaluated the reproductive performance of sows after recovery from ASF and the effect of passive immunity on the course of ASF in young pigs. Six sows were inoculated with an ASFV Dominican Republic isolate, allowed to recover and bred. Vaginal, nasal and oral swab samples and blood samples were collected during pregnancy, at farrowing and during lactation and evaluated for the presence of ASFV. Pre-colostral and colostral blood samples were collected from the piglets and tested for anti-ASFV antibodies by ELISA.

Of the six sows that were bred after recovery, one conceived soon after the introduction of boars, four conceived at 368-419 DPI, and one sow did not conceive at all. During the periparturient period, ASFV was not isolated from any swab or blood samples in any sow. 170 frozen tissue sections from 25 piglets born of four sows were examined via immunofluorescence for the presence of ASFV antigen, and it was not detected. The tissue cultures were also negative for ASFV (Schlafer *et al.*, 1984a).

The anti-ASFV antibody levels of sows did not change significantly during farrowing and lactation (Schlafer *et al.*, 1984a). One litter of piglets was nursed with a sow after birth. The piglets did not have detectable pre-colostral anti-ASFV antibodies, but they were detectable two days after birth. The antibody levels declined gradually over the following seven weeks (Schlafer *et al.*, 1984a). At seven weeks of age, the litter (eight pigs) was oronasally inoculated with the same ASFV isolate as their dam. Seven piglets developed increased rectal temperature at 13 DPI. Four pigs became viremic at 5 DPI and the other three at 13-20 DPI. One pig did not develop a detectable viremia or an antibody response (Schlafer *et al.*, 1984a).

The litter of pigs was inoculated again at 105 days of age, with the same ASFV isolate as before. The pig that had been asymptomatic at first inoculation developed clinical signs of ASF and viremia (Schlafer *et al.*, 1984a). The other seven pigs developed viremia, but they

did not develop fever during the two weeks following inoculation. Four pigs were killed at 119 days of age, and 26 tissue samples from each were cultured for ASFV. ASFV was isolated from many tissue samples in two pigs, but in the other two, virus was cultured only in the sternal lymph nodes and tonsils (Schlafer *et al.*, 1984a).

The effect of passively derived anti-ASFV antibodies in neonatal pigs was more specifically investigated in another study by Schlafer *et al.* (1984b). 30 piglets were acquired via hysterotomy performed on specific pathogen-free sows and assigned to five study groups at 8-11 days of age: colostrum-deprived and fed with a commercial milk replacer (I); fed with colostrum from a normal sow two hours after birth and fed with a commercial milk replacer thereafter (II); fed colostrum from a sow that had recovered from experimentally-induced ASF (III); given ASF-antiserum from a hyperimmunized pig (IV); non-inoculated control group (V). The neonatal pigs were subsequently inoculated with and ASFV Dominican Republic isolate and disease progression was monitored by recording clinical sings, viremia titers and antibody response, as well as necropsy performed on animals that succumbed to ASF.

The piglets in group III and IV displayed a markedly altered course of disease – they had a delayed onset of clinical signs, lower viremia titers and a significantly higher, survival rate than piglets in group I and II (Schlafer *et al.*, 1984b). The piglets in group I and II were dead by 16 DPI (Table 2).

Table 2. Mean time of onset of clinical signs after inoculation, mean viremia titers at 4 days after inoculation, individual and mean days of death in four treatment groups (Schlafer *et al.,* 1984b)

Group, number of pigs in the group, n	Age at inoculation, days	Onset of clinical signs, DPI	Viremia titer at 4 DPI, log10 Had50/ml	Individual days of death, DPI	Mean day of death, DPI
I – colostrum-deprived (n = 7)	8 to 11	4.2 ± 1.5	7.9 ± 0.48	4; 5; 6; 7; 7; 8	6.3 ± 1.4
II – fed colostrum from a normal sow (n = 8)	8 to 11	4.1 ± 0.7	7.6 ± 0.60	5; 6; 6; 6; 7; 8; 11; 16	8.1 ± 3.7
III – fed colostrum from an ASF-recovered sow (n = 5)	8 to 11	5.8 ± 1.5	3.4 ± 2.60	139; 151	NA*
IV – given serum from a hyperimmunized pig (n = 5)	8 to 11	7.4 ± 1.8	3.0 ± 1.10	40; 65; 67	NA*

Notes:

- DPI - days post-inoculation;
- n - number of pigs in the group;
- NA - not applicable;
- * several pigs in groups III and IV were still alive at 184 DPI

In comparison, only one pig in group III had died by 38 DPI. It should be noted, however, that passively immunized piglets (groups III and IV) did develop an active immune response to ASF, but it was delayed compared to piglets that were not immunized (Schlafer *et al.*, 1984b). Two pigs from group III died at DPI 139 and 151, respectively (Table 2). Three pigs in group IV developed recurrent viremia and fever and died at 40-67 DPI. The remaining pigs in groups III and IV were still alive at 184 DPI (Schlafer *et al.*, 1984b).

2. MATERIALS AND METHODS

2.1. The study population

The dataset used in this study was created in Microsoft Excel using the official African swine fever surveillance data in Estonia, which was collected by the Estonian Veterinary and Food Board. This dataset included data of wild boar found dead, hunted, destroyed as sick or killed in a road traffic accident in the time period of 2014 until the end of 2019. The ASF status of each animal had been determined by means of PCR and serology, and the results thereof had been indicated. For each animal, the date of collection and the location (county) where it was found had been identified. Each animal had been grouped to one of the following age classes: 0 (age unknown); 1 (animals less than one year of age, <1y); 2 (animals 1-2 years of age, 1-2y); 3 (animals more than two years of age, >2y). The animals whose age was unknown were excluded from further analysis.

2.2. Data management

A county-level dataset was created in Microsoft Excel, where data were summarised by calendar quarters. Each date of when the animal was found was assigned to one of the following calendar quarters, based on the month when it was found: Q1 (January, February, March); Q2 (April, May, June); Q3 (July, August, September); Q4 (October, November, December).

For the purposes of this study, animals were grouped into two age classes: less than one year of age (young wild boar, <1y) and animals one year of age or older (adult wild boar, ≥1y). The reason for this was that in the data from 2014, the animals were grouped in age classes this way, and the rest of the dataset had to be made uniform to perform calculations. For statistical analysis, the data from 2015-2019 and the original age classes of the initial dataset were used.

Wild boar were assigned to seropositive, seronegative, ASFV-positive and ASFV-negative groups. To do this, a binary system was used – "1" denoted seropositive and ASFV-positive and "0" denoted seronegative and ASFV-negative animals, respectively.

To compare the disease dynamics on a regional level, Estonia (Figure 7) was divided into three regions depending on the time of the disease incursion as follows: South-East (Võrumaa, Valgamaa, Viljandimaa, Põlvamaa, Tartumaa) – disease incursion in 2014 or early 2015; North-east (Ida-Virumaa, Lääne-Virumaa, Jõgevamaa, Järvamaa) – introduction in Ida-Virumaa in 2014, but the main spread occurred in late 2015; West (Harjumaa, Läänemaa, Raplamaa, Pärnumaa, Saaremaa) – disease incursion in 2016. The county of Hiiumaa was excluded from the analysis, as it remained disease-free over the whole time period.

Figure 7. Map of the counties in Estonia and their division into regions – South-east (blue outline), North-east (black outline) and West (red outline) (adapted from Wikipedia).

2.3. Data analysis

The data analysis detailed in this section was performed using Microsoft Excel. The tables and charts that illustrate the results thereof were generated using the same software.

To begin analysis of data, the number of seropositive, serologically tested, ASFV-positive and PCR-tested wild boar, respectively, was determined in each age class per year on a regional level (Table 3). These numbers were also determined per calendar quarter in these regions.

Table 3. The number of PCR-positive, PCR-tested, seropositive and serologically tested wild boar per year in each region in Estonia in 2014-2019

Region	**Year**	**PCR-tested**		**<1y, serologically tested**		**≥1y, serologically tested**	
		positive	**total tested**	**positive**	**total tested**	**positive**	**total tested**
North-east	2014	3	71	1	12	0	53
	2015	246	2 836	37	1 097	25	1 684
	2016	434	4 340	82	1 778	85	2 245
	2017	45	887	21	316	44	5 22
	2018	5	850	6	340	30	495
	2019	4	673	1	250	12	407
	Total	**737**	**9 657**	**148**	**3 793**	**196**	**5 406**
South-east	2014	69	525	1	108	2	318
	2015	728	4 669	57	1 535	44	2 993
	2016	315	3 665	79	1 332	78	1 150
	2017	28	684	10	282	39	358
	2018	5	1 158	5	535	30	584
	2019	0	1 340	2	673	16	633
	Total	**1 145**	**12 041**	**154**	**4 465**	**209**	**6 036**
West	2015	29	2 031	4	597	0	1 351
	2016	530	7 815	57	3 356	35	4 839
	2017	518	7 226	107	2 661	155	4 228
	2018	48	2 185	37	777	144	1 371
	2019	2	1 802	7	731	39	1 049
	Total	**1 127**	**21 059**	**212**	**8 122**	**373**	**12 838**
Grand total		**3 009**	**42 757**	**514**	**16 380**	**778**	**24 280**

Notes:

- PCR – polymerase chain reaction;
- <1y – wild boar less than one year of age;
- ≥1y – wild boar one year of age or older

Using the derived data, the seroprevalence (formula 2.2.1.) and viroprevalence (formula 2.2.2.) were calculated per year and per calendar quarter, respectively, on a county level.

Then, the average viroprevalence and seroprevalence of each region were determined per calendar quarter using the Pivot Table function in Microsoft Excel and displayed using Pivot Chart.

Formula for calculating seroprevalence (Thrusfield, 2005):

$$P(sero) = \frac{S}{N(sero)}, \quad (2.2.1.)$$

where

P (sero) – seroprevalence;

S - number of seropositive wild boar in an age class in a calendar quarter in a county;

N (sero) – number of serologically tested wild boar in an age class in a calendar quarter in a county.

Formula for calculating viroprevalence (Thrusfield, 2005):

$$P(viro) = \frac{V}{N\ (viro)}, \quad (2.2.2.)$$

where

P (viro) – viroprevalence;

V – number of PCR-positive wild boar in an age class in a calendar quarter in a county;

N (viro) – number of PCR-tested wild boar in an age class in a calendar quarter in a county.

For statistical analysis, prevalence ratios (PR) per year on a regional level were calculated for age classes. To do this, the original classes of the initial dataset were used. The average seroprevalence per year of young wild boar (<1y) was divided by that of the 1-2y and >2y age classes, respectively (formula 2.2.3. and 2.2.4.).

Formula for calculating prevalence ratio (Dohoo *et al.*, 2003):

$$PR = \frac{P_{<1y}}{P_{1-2y}}, \quad (2.2.3.)$$

where

PR – prevalence ratio;

$P_{<1y}$ – average seroprevalence of wild boar less than one year of age in a year in a region;

$P_{1\text{-}2y}$ – average seroprevalence of wild boar 1-2 years of age in a year in a region.

Formula for calculating prevalence ratio (Dohoo *et al.*, 2003)

$$PR = \frac{P_{<1y}}{P_{>2y}}, \quad (2.2.4.)$$

Where

PR – prevalence ratio;

$P_{<1y}$ – average seroprevalence of wild boar less than one year of age in a year in a region;

$P_{>2y}$ – average seroprevalence of wild boar more than 2 years of age in a year in a region.

To estimate the duration of the ASF epidemic in Estonia, for every county, the duration of observations of ASFV-positive, seropositive animals in all age classes and young seropositive animals (<1y age class), respectively, was counted by calendar quarters. In addition, the time of observing young seropositive animals after the last detection of an ASFV-positive animal was defined using the same method. Frequency distributions were illustrated as histograms using the Data Analysis ToolPak in Microsoft Excel. The dynamics of average viroprevalence and seroprevalence in the <1y and ≥1y age class, as well as the number of seropositive animals in the <1y age class on the country level was illustrated in a graph generated in Microsoft Excel.

2.4. Statistical analysis

A two-tailed Z-test with the desired significance level 0.05 was performed in order to determine if the differences in the seroprevalence of young wild boar (<1y age class) compared to that of the other age classes is statistically significant. The open-access software Epitools was used (https://epitools.ausvet.com.au/ztesttwo) for this analysis.

Correlation analysis was performed to assess associations between the occurrence of ASFV-positive animals or viroprevalence and seroprevalence in different age classes. Pearson correlation coefficients were calculated using the XLSTAT software (https://www.xlstat.com/en/).

Descriptive statistics on the county level were performed using the XLSTAT software (https://www.xlstat.com/en/) on the counts of calendar quarters of the duration of observations of ASFV-positive animals, seropositive animals in all age classes, young

seropositive animals (<1y age class), and time from the detection of the last ASFV-positive animal to the last young seropositive animal.

3. RESULTS

3.1. The dynamics of viroprevalence and seroprevalence of African swine fever on a regional level in Estonia

The average viroprevalence and seroprevalence per year in each region in Estonia are presented in Table 4. When considering the average viroprevalence, the spread of ASFV is more gradual in the North-east and West regions, compared to the South-East. The average viroprevalence on the first year of the epidemic in the North-east, South-east and West regions is 4,23%, 12,23% and 0,87%, respectively. In the North-east and South-east, the first detections of ASFV were made in 2014, while in the West this occurred later, in 2015.

Table 4. Average viroprevalence and seroprevalence per year in each region in Estonia during the African swine fever epidemic in 2014-2019

Region	Year	Average viroprevalence, %	Average seroprevalence in <1y age class, %	Average seroprevalence in ≥1y age class, %
North-east	**2014**	4,23%	8,33%	0,00%
	2015	3,76%	2,56%	1,31%
	2016	15,00%	14,13%	9,75%
	2017	4,60%	21,14%	12,62%
	2018	0,36%	1,04%	7,30%
	2019	0,17%	0,19%	3,68%
South-east	**2014**	12,23%	1,00%	0,70%
	2015	13,36%	3,62%	1,65%
	2016	11,54%	11,74%	11,64%
	2017	3,39%	5,38%	11,29%
	2018	0,79%	1,75%	3,22%
	2019	0,00%	0,07%	2,64%
West	**2015**	0,87%	0,28%	0,00%
	2016	10,53%	1,76%	0,77%
	2017	9,64%	10,83%	7,01%
	2018	1,51%	5,65%	14,03%
	2019	0,11%	3,05%	5,10%

Notes:

- **<**1y – wild boar less than one year of age;
- ≥1y – wild boar one year of age or older

When considering the average seroprevalence per year in the <1y and ≥1y age classes (Table 4), it becomes apparent that in all three regions, over the first three years of the ASF epidemic the average seroprevalence increases more in the <1y age class compared to the ≥1y age class. At its highest, the average seroprevalence in the North-east and South-east is higher in the <1y age class than in ≥1y wild boar – 21,14% and 12,62%, respectively, in the North-east and 11,74% and 11,64%, respectively, in the South-east. In the West, the highest average seroprevalence in the <1y age class was 10,83%, while in the ≥1y age class it was 14,03%. The highest average seroprevalence in both age classes occurred in the same year (2016) in both North-east and South-east, but in the West the highest average seroprevalence in the <1y age class was in 2017 and in the ≥1y age class it was in 2018.

In all three regions, the highest average viroprevalence per year is followed by the highest average seroprevalence in the <1y age class in the following year (Table 4). In the North-east and South-east, this is also true for the ≥1y age class. In the West, the highest average seroprevalence in the ≥1y age class (14,03%) occurred in 2018, while the highest average viroprevalence (10,53%) was in 2016.

The dynamics of the average viroprevalence and seroprevalence over the duration of the ASF epidemic are illustrated for each region in Estonia – South-east (Figure 8), North-east (Figure 9) and West (Figure 10). Here, the changes in these dynamics are illustrated over calendar quarters, so that more minute details become apparent.

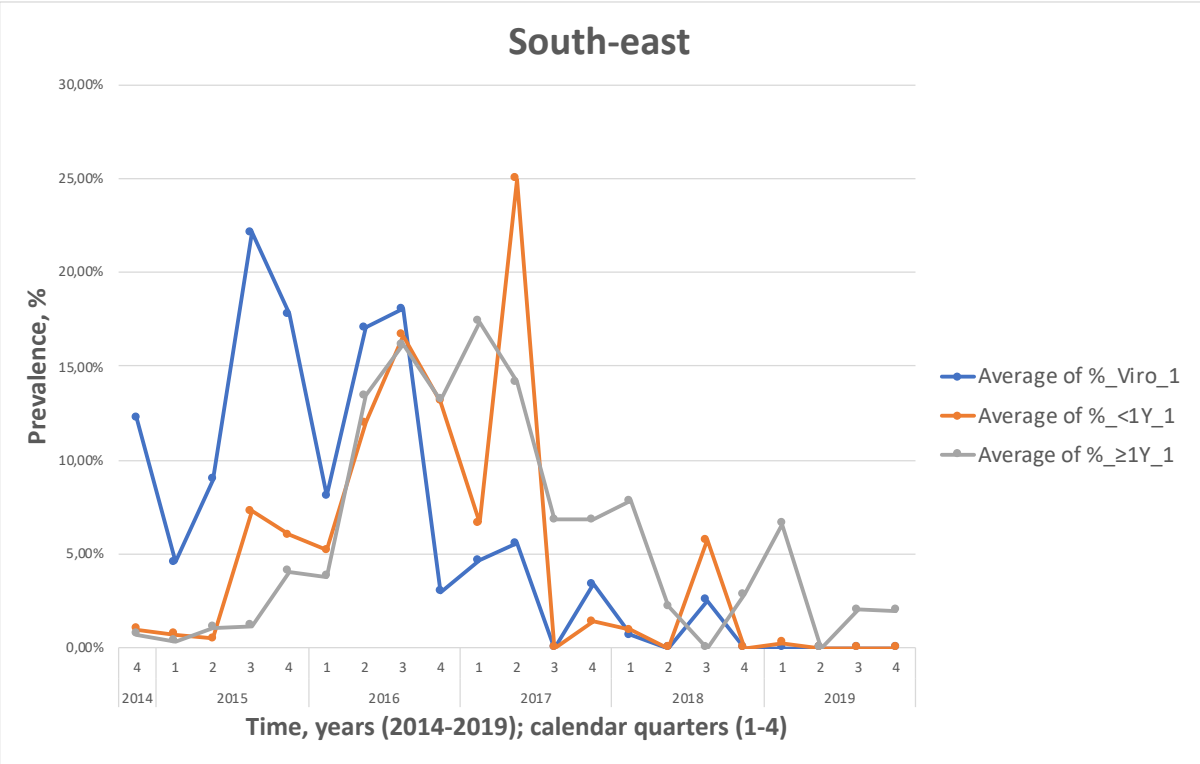

Figure 8. Average viroprevalence (blue colour) and seroprevalence (<1y age class – orange colour; ≥1y age class – grey colour) of African swine fever per calendar quarter in the South-eastern region of Estonia in 2014-2019. The viroprevalence and seroprevalence are expressed as a percentage (%). %_Viro_1 – viroprevalence; %_<1Y_1 – seroprevalence in wild boar less than one year of age; %_≥1Y_1 – seroprevalence in wild boar one year of age or older.

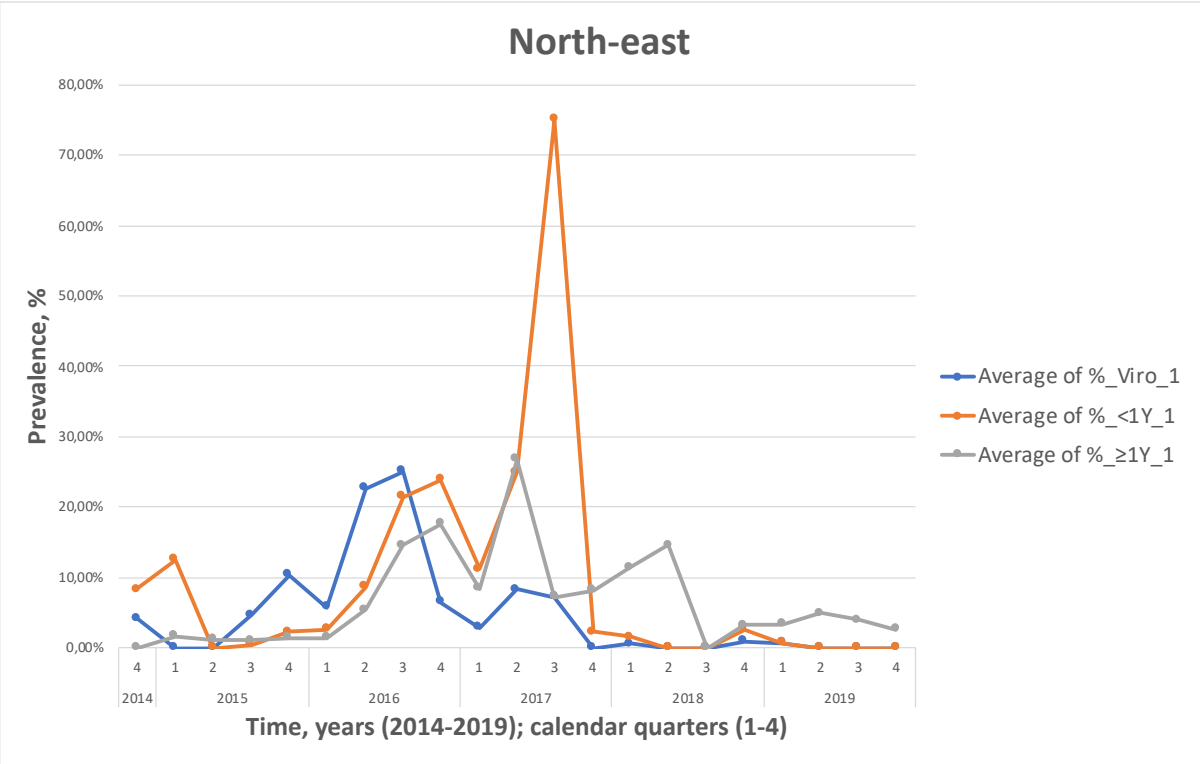

Figure 9. Average viroprevalence (blue colour) and seroprevalence (<1y age class – orange colour; ≥1y age class – grey colour) of African swine fever per calendar quarter in the North-eastern region of Estonia in 2014-2019. The viroprevalence and seroprevalence are expressed as a percentage (%). %_Viro_1 – viroprevalence; %_<1Y_1 – seroprevalence in wild boar less than one year of age; %_≥1Y_1 – seroprevalence in wild boar one year of age or older.

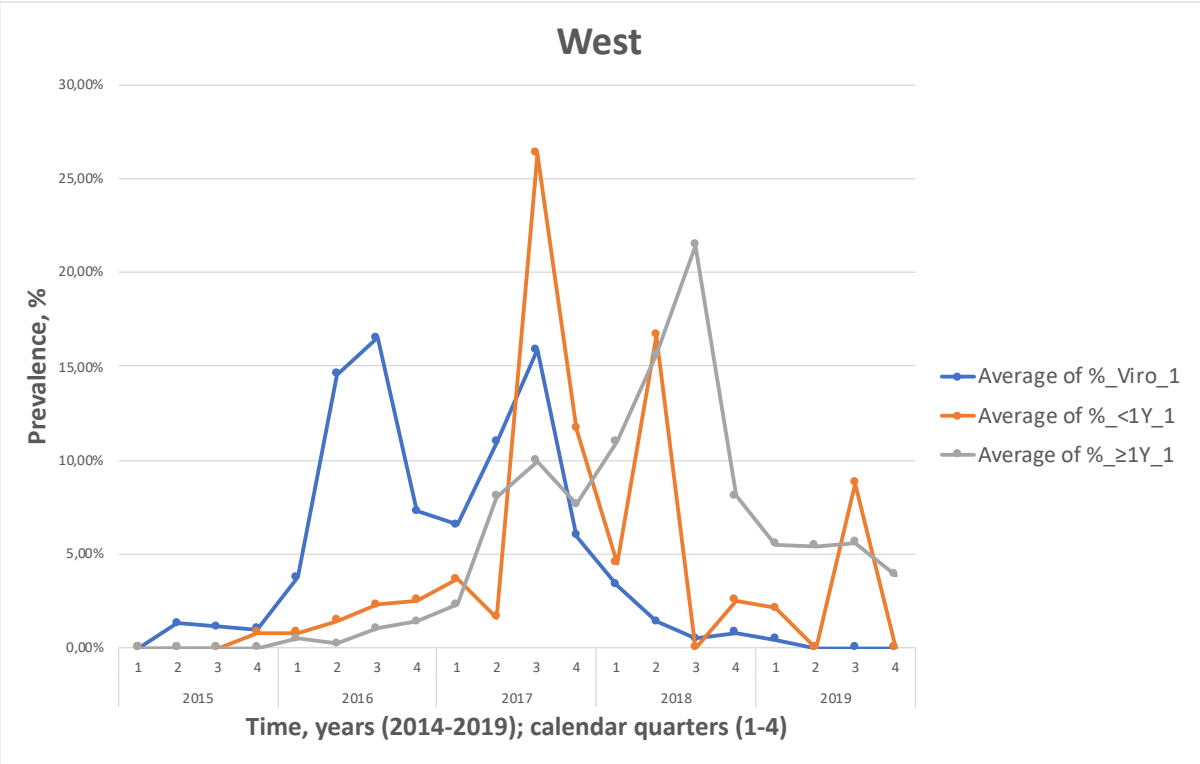

Figure 10: Average viroprevalence (blue colour) and seroprevalence (<1y age class – orange colour; ≥1y age class – grey colour) of African swine fever per calendar quarter in the Western region of Estonia in 2014-2019. The viroprevalence and seroprevalence are expressed as a percentage (%). %_Viro_1 – viroprevalence; %_<1Y_1 – seroprevalence in wild boar less than one year of age; %_≥1Y_1 – seroprevalence in wild boar one year of age or older.

In Figures 8-10, as in Table 4, it is apparent that, at the start of the ASF epidemic, the average viroprevalence increases more gradually in the North-east and West (Figures 9 and 10, respectively), compared to the South-east (Figure 8). In the South-east, the highest average viroprevalence was 22,11% in the 3rd calendar quarter in 2015, followed by a sharp decrease and a subsequent sharp rise to 18,07% a year later. After this, the average viroprevalence in this region decreases gradually to 0,00% in the 4th calendar quarter in 2018. In the North-east (Figure 9), the viroprevalence increases gradually from the first detection of ASFV in 2014 to a peak of 25,05% in the 3rd calendar quarter in 2016. Then, it decreases gradually to 0,00% from the 2nd calendar quarter in 2019 onwards. In the West (Figure 10), there are two peaks in viroprevalence – 16,52% in the 3rd quarter in 2016 and 15,86% in the 3rd quarter in 2017. It gradually decreases to 0,00% in the 2nd quarter in 2019.

In the South-east (Figure 8), North-east (Figure 9) and West (Figure 10), there is a pronounced peak in the average seroprevalence in the <1y age class in the 2nd (South-east) and 3rd (North-east and West) calendar quarter in 2017. The highest average seroprevalence in the <1y age class is 25,00%, 75,00% and 25,63% in the South-east, North-east and West, respectively. This is followed by a sharp decrease in the average seroprevalence in this age class in the following calendar quarter. From the 1st calendar quarter in 2018 onwards, no more sharp rises of this magnitude are seen in the South-east and North-east. In the West, there is a pronounced increase in the average seroprevalence in the <1y age class in the 2nd calendar quarter in 2018, but it was not as high as that in 2017. In, 2019, the average seroprevalence in the <1y age class is relatively low and stable in the South-east and North-east, while in the West, there is an increase from 0,00% in the 2nd calendar quarter to 8,79% in the 3rd quarter, before decreasing to 0,00% again in the 4th quarter.

The dynamics of the average seroprevalence in the ≥1y age class are more variable in the three regions compared to the <1y age class. In the South-east (Figure 8), it increases gradually in 2014 and 2015, then more sharply in 2016, and reaching a peak of 17,37% in the 1st quarter in 2017. After that, there is a gradual decrease to 0,00% in the 3rd quarter in 2018 and an increase to 6,58% in the 1st quarter in 2019. There is a decrease to 0,00% again in the following quarter and an increase to 2,00% in the 3rd and 4th quarter. In the North-east, (Figure 9), the average seroprevalence in the ≥1y age class increases relatively gradually until it reaches its highest point of 26,78% in the 2nd quarter in 2017. It decreases gradually to 0,00% in the 3rd quarter in 2018 and increases slightly to 3,24% in the next quarter and maintains a relatively stable level over 2019. The average seroprevalence in the ≥1y age class in the Western region (Figure 10) is 0,00% over the duration of 2015, followed by a slow and gradual increase to 1,37% in 2016. After that, the average seroprevalence in this age class increases more sharply until reaching its highest point of 21,44% in the 3rd quarter in 2018. After that, there is a relatively sharp decrease, but unlike in the North-east and South-east, it does not reach 0,00%. Instead, there is a gradual decrease to 3,90% in the 4th quarter in 2019.

In the South-east (Figure 8), the highest peak in the average viroprevalence is followed by a more pronounced increase in the average seroprevalence in the <1y and ≥1y age classes two calendar quarters later. There is a second peak in average viroprevalence in the 3rd calendar

quarter in 2016, which is followed by a peak in average seroprevalence in the ≥1y age class and the highest peak in the <1y age class two and three calendar quarters later, respectively. As the average seroprevalence in both age classes starts to decrease in 2017, the decrease of the average seroprevalence in the ≥1y age class is more gradual than that of the <1y age class. After the 3rd calendar quarter in 2017, the average seroprevalence tends to be higher in the ≥1y age class compared to the <1y age class.

The highest average viroprevalence in the North-east (Figure 9) precedes the highest average seroprevalence in the ≥1y age class and the <1y age class by three and four calendar quarters, respectively. In this region, as in the South-east, the average seroprevalence decreases in 2017 in both age classes and that in the <1y age class age class decreases more sharply compared to the ≥1y age class. In addition, after this decrease, the average seroprevalence tends to be higher in the ≥1y age class, similarly to the South-east.

The dynamics of viroprevalence and seroprevalence differ in the West (Figure 10) compared to the South-east and North-east. There is a pronounced peak in the average viroprevalence in the 3rd quarter in 2015 and 2016, respectively. The peak in 2015 precedes the rise in average seroprevalence in the <1y age class and ≥1y age class by three and four calendar quarters, respectively. The peak in average viroprevalence in 2016 coincides with a sharper increase in the average seroprevalence in the ≥1y age class and the highest peak in the <1y age class. It precedes a second sharp rise in the average seroprevalence in the <1y age class by three calendar quarters and the highest peak in the ≥1y age class by four quarters. In contrast to the average seroprevalence in the <1y age class, that of the ≥1y does not decrease in 2017 but continues to rise to its peak in 2018. After that, as in the South-east and North-east, the average seroprevalence in the ≥1y age class tends to be higher than in the <1y age class.

3.2. Comparison of average seroprevalence in young wild boar to the average seroprevalence in older age classes

The prevalence ratios (PR) of the <1y age class compared to the 1-2y and >2y age class, respectively, are presented in Table 5. In the North-east, in 2015, 2016 and 2017, the average

seroprevalence in the <1y age class is higher than in the 1-2y age class, and the PR is statistically significant (PR=3,76; 1,38 and 2,90, respectively, with a significance level 0,05). In 2018 and 2019, the average seroprevalence is higher in the 1-2y age class compared to that in the <1y age class. This finding is statistically significant in 2018 (PR=0,21) but not 2019 (PR=0,11). There are similar findings when comparing the average seroprevalence of the <1y age class to that of the >2y age class. When comparing the average seroprevalence in these age classes, that of the <1y age class is relatively higher in 2015, 2016 and 2017, but only the PR in 2017 (PR=1,87) is statistically significant. The average seroprevalence in the >2y age class becomes higher than that of the <1y age class in 2018 and 2019 in the North-east (PR=0,11 and 0,04, respectively), which is statistically significant.

Table 5. Comparison of average yearly seroprevalence (prevalence ratios) of African swine fever in wild boar less than one year of age (<1y) and wild boar 1-2 years of age (1-2y) and more than two years of age (>2y), respectively, in each region in Estonia in 2015-2019. Statistically significant prevalence ratios (significance level 0,05), as determined by the two-tailed Z-test, are indicated with an asterisk

Region	Year	PR <1y/1-2y	PR <1y/>2y
North-east	**2015**	3,76*	1,35
	2016	1,38*	1,87*
	2017	2,90*	1,12
	2018	0,21*	0,11*
	2019	0,11	0,04*
South-east	**2015**	1,92*	2,13*
	2016	0,97	1,05
	2017	0,78	0,32*
	2018	0,57	0,47
	2019	0,13	0,01*
West	**2015**	NA	NA
	2016	1,72*	2,85*
	2017	1,12	2,14*
	2018	0,41*	0,37*
	2019	1,72	0,41*

Notes:

- PR – prevalence ratio;
- <1y – wild boar less than one year of age;
- 1-2y – wild boar 1-2 years of age;
- >2y – wild boar more than two years of age;
- * - statistically significant prevalence ratio (significance level 0,05);
- NA – not applicable

In the South-east (Table 5), the average seroprevalence is higher in the <1y age class compared to that of the 1-2y and >2y age class in 2015 (PR=1,92 and 2,13, respectively,

significance level 0,05). These PR are statistically significant. Starting from 2016, the average seroprevalence in the 1-2y age class becomes higher than that of <1y age class, but these PR are not statistically significant. This is also true for the <1y age class compared to the >2y age class starting from 2017, but here the PR for 2017 and 2019 (PR=0,32 and 0,01, respectively) are statistically significant.

In the West (Table 5), the PR could not be calculated for 2015, as the average seroprevalence in the 1-2y and >2y age classes was 0,00%. The average seroprevalence in the <1y age class is higher than that of the 1-2y age class in 2016 and 2017, however only the PR in 2016 (PR=1,72, significance level 0,05) is statistically significant. In 2018, the average seroprevalence becomes higher (PR=0,41) in the 1-2y age class. In 2019, the seroprevalence in the <1y age class becomes higher again, but this is not statistically significant. When comparing the average seroprevalence in the <1y age class and >2y age class, it is higher in the <1y age class in 2016 and 2017 (PR=2,85; 2,14, respectively). In 2018 and 2019, the average seroprevalence in the >2y age class becomes higher (PR=0,37; 0,41, respectively). All PR for the <1y age class and >2y age class in 2016-2019 are statistically significant.

3.3. Correlation analysis

Pearson correlation coefficients (r) and their corresponding p-values are presented in Table 6. The relationship of the variables in Table 6 is illustrated as a series of scatter plots in Figure 11 and 12. Table 6 shows that there is a statistically significant positive correlation between the number of ASFV-positive animals and the number of seropositive animals in the <1y age class (r=0,565; p=<0,0001) and the number of seropositive animals in the 1-2y age class (r=0,296; p=0,0002). There is a statistically significant negative correlation between the number of ASFV-positive animals and the average seroprevalence in the >2y age class (r=-0,286; p=0,0003).

Table 6. Pearson correlation coefficients (r) and their corresponding p-values of the occurrence of ASFV-positive animals, viroprevalence and seropositive animals and seroprevalence in different age classes. Statistically significant (p <0,05) correlation coefficients are indicated with an asterisk

Variables	ASFV-positive, n		Viroprevalence, %	
	correlation coefficient, r	p-value	correlation coefficient, r	p-value
Seropositive <1y, n	0,565*	<0,0001	0,102	0,21
Seroprevalence <1y, %	-0,088	0,28	0,303*	0,0001
Seropositive, 1-2y, n	0,296*	0,0002	0,150	0,06
Seroprevalence, 1-2y, %	-0,071	0,38	0,198*	0,01
Seropositive, >2y, n	-0,047	0,56	-0,161*	0,05
Seropositive, >2y, %	-0,286*	0,0003	-0,029	0,72

Notes:

- **<**1y – wild boar aged less than one year of age;
- 1-2y – wild boar 1-2 years of age;
- >2y – wild boar more than two years of age;
- * - statistically significant correlation coefficient (values are different from 0 with a significance level 0.05);
- r – Pearson correlation coefficient;
- n – number of animals

There is a statistically significant positive correlation between the average ASF viroprevalence and the average seroprevalence in the <1y age class (Table 6 - r=0,030; p=0,0001) and the 1-2y age class (r=0,198; p=0,01). The average viroprevalence has a statistically significant negative correlation with the number of seropositive animals in the >2y age class (r=-0,161; p=0,05).

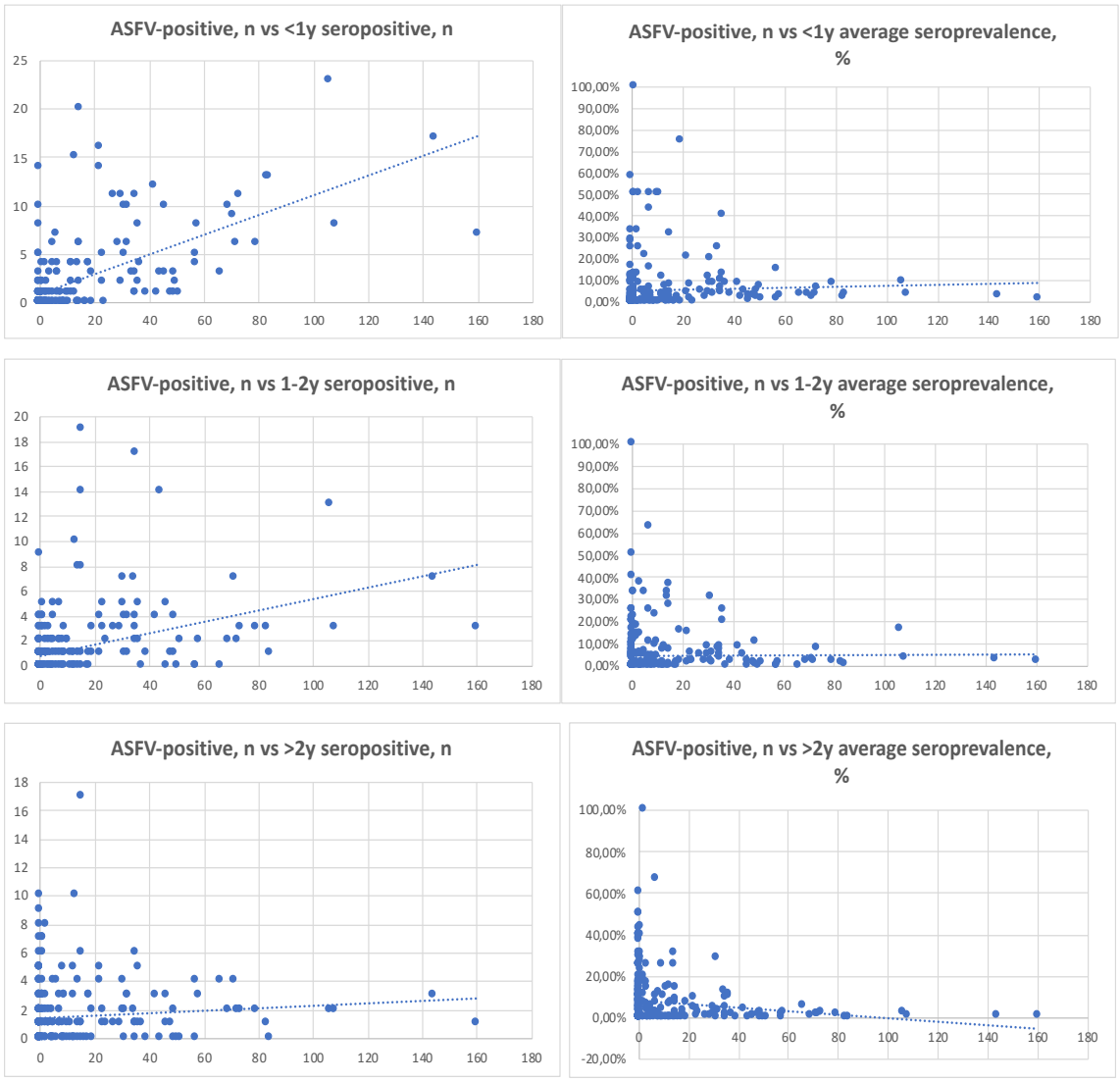

Figure 11. Scatter plots that illustrate correlation of the following variables: the number of ASFV-positive animals (n) versus the number of seropositive animals (n) and the average seroprevalence (%) in the age classes of wild boar less than one year of age (<1y), wild boar 1-2 years of age (1-2y) and wild boar more than two years of age (>2y), respectively. These variables are calculated per calendar quarter on a county level in Estonia in 2014-2019. The viroprevalence and seroprevalence are expressed as a percentage (%). ASFV – African swine fever virus; <1y – wild boar less than one year of age; 1-2y – wild boar 1-2 years of age; >2y – wild boar older than two years of age; n – number of animals.

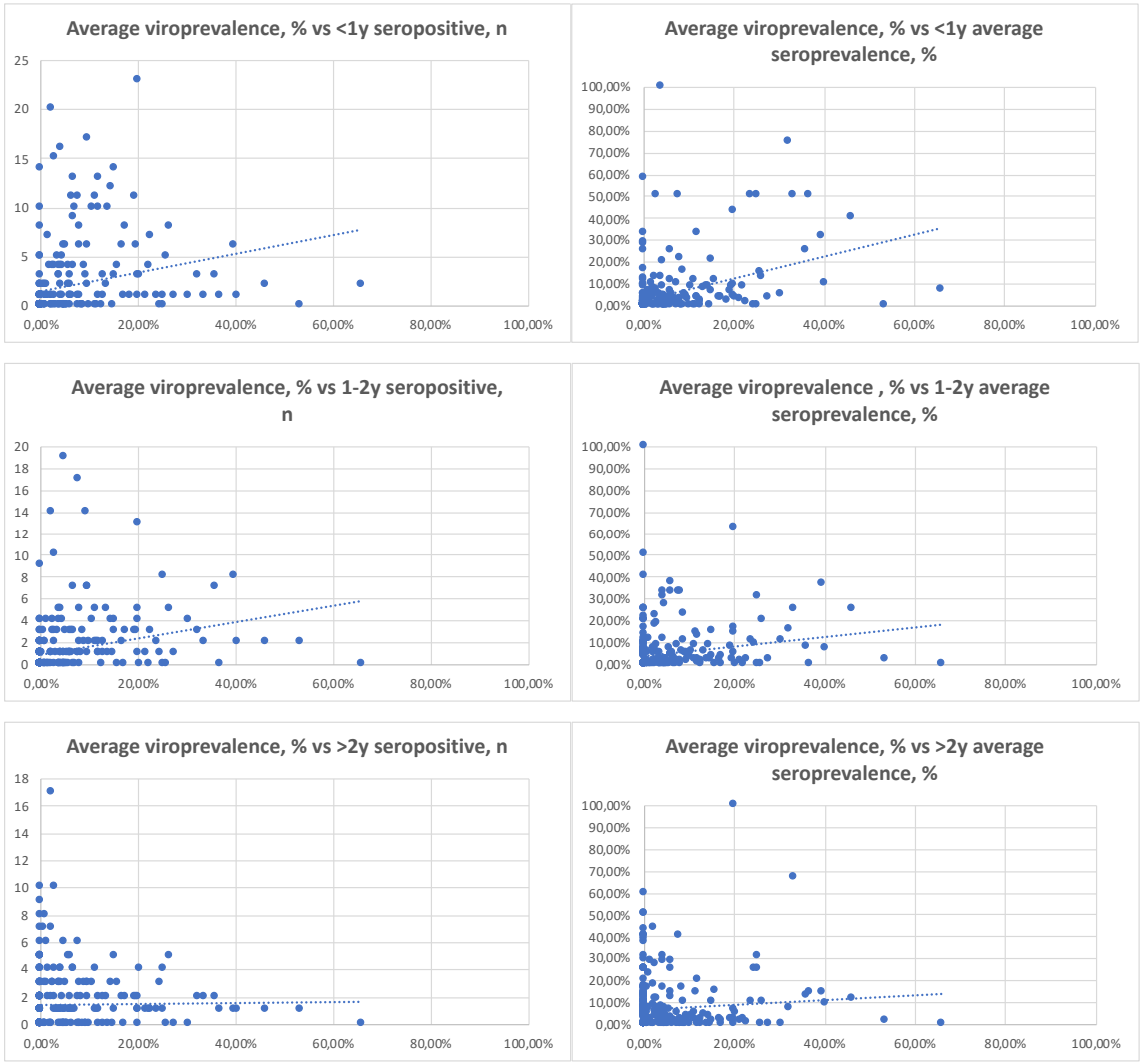

Figure 12. Scatter plots that illustrate the correlation of the following variables: average viroprevalence (%) of African swine fever versus the number of seropositive animals (n) and the average seroprevalence (%) in the age classes of wild boar less than one year of age (<1y), wild boar 1-2 years of age (1-2y) and wild boar more than two years of age (>2y), respectively. These variables are calculated per calendar quarter on a county level in Estonia in 2014-2019. The viroprevalence and seroprevalence are expressed as a percentage (%). <1y – wild boar less than one year of age; 1-2y – wild boar 1-2 years of age; >2y – wild boar older than two years of age; n – number of animals.

3.4. Duration of observation of African swine fever virus-positive animals, seropositive animals and the epidemic in Estonia

The dynamics of average viroprevalence and seroprevalence in the <1y and ≥1y age class, respectively, as well as the sum of seropositive animals in the <1y age class are illustrated in Figure 13. These data are presented on a country level in Estonia over the duration of the ASF epidemic (i.e. 2014-2019). The time is expressed as calendar quarters. Here, as in Figures 8-10, where these dynamics are illustrated on a regional level, the average seroprevalence in the ≥1y age class tends to be higher than that of the <1y age class towards the end of the epidemic (i.e. from the 11th calendar quarter onwards). The highest peak in average viroprevalence (5th calendar quarter) is followed by the highest peak in average seroprevalence in both age classes three calendar quarters later. There is a second, lower peak in average seroprevalence in the 8th calendar quarter, which is followed by a sharper rise in the average seroprevalence in the ≥1y and <1y age class three and four calendar quarters later, respectively. On the country level, the average seroprevalence of wild boar in the ≥1y age class does not fall to 0,00%, as that of the <1y age class does, but maintains a steady low level, and increases to 5,00% at the end of the study period. The average viroprevalence and seroprevalence in the <1y age class fall to 0,00% starting from the 19th calendar quarter.

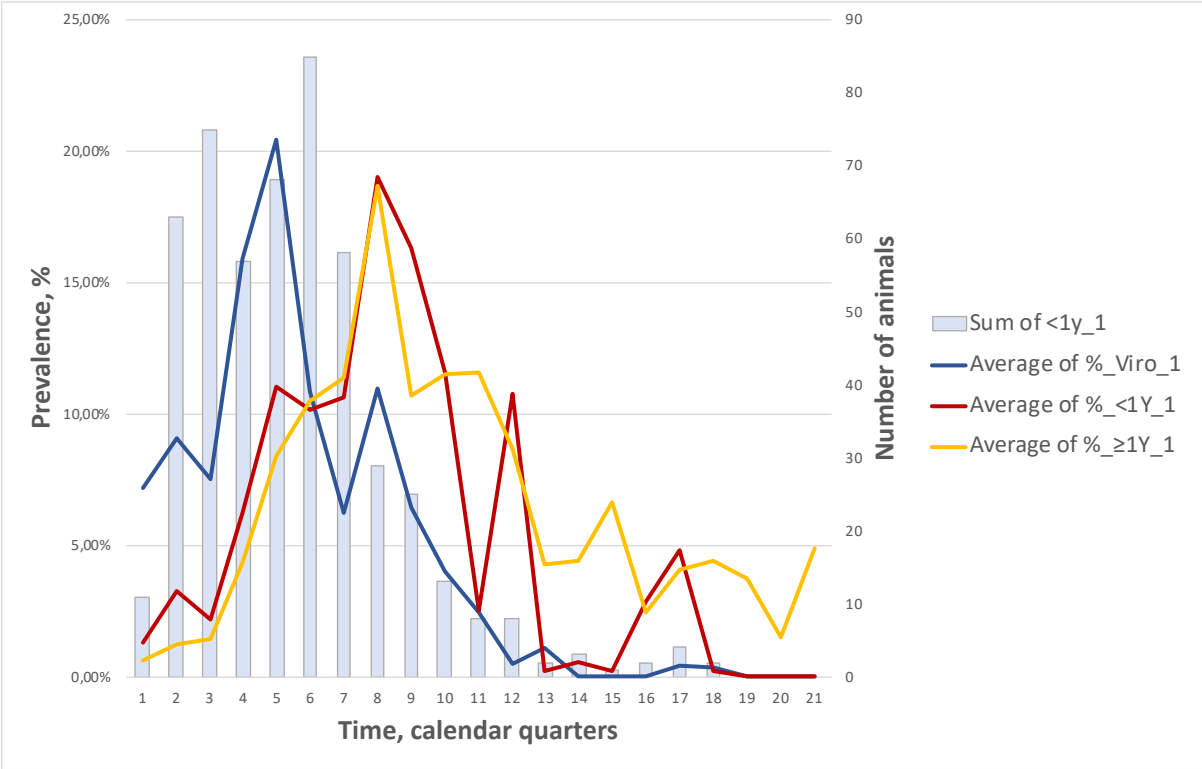

Figure 13. Dynamics of average viroprevalence (dark blue colour) and seroprevalence (<1y age class – red colour; ≥1y age class – yellow colour) and the sum of seropositive wild boar less than one year of age (light blue colour bars) on the country level in Estonia during the African swine fever epidemic (2014-2019). Time is expressed as calendar quarters. <1y_1 – number of seropositive animals in wild boar less than one year of age; %_Viro_1 – viroprevalence; %_<1y_1 – seroprevalence in wild boar less than one year of age; %_≥1y_1 – seroprevalence in wild boar one year of age or older.

Frequency distributions of time during which ASFV-positive and seropositive animals were observed on the county level are illustrated in Figure 14, with a focus on seropositive animals in the <1y age class. The frequency distribution of time between the detection of the last ASFV-positive animal to the detection of the last seropositive animal in the <1 age class on the county level is illustrated in Figure 15. The results of descriptive statistics of the data illustrated in Figure 14 and 15 are presented in Table 7. In total, data of time was analysed in 14 counties.

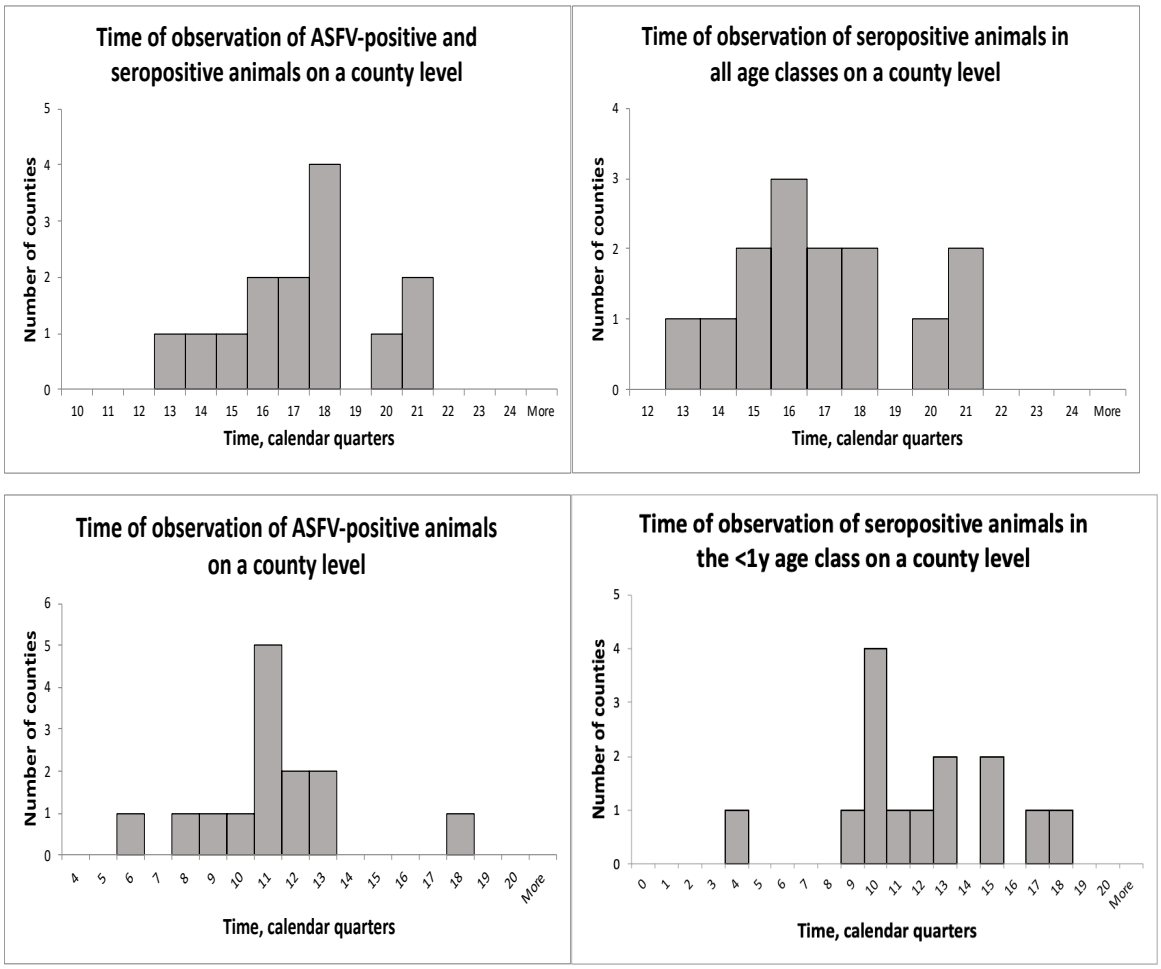

Figure 14. Frequency distributions of the time of observation of ASFV-positive and seropositive animals, with a focus on seropositive animals in the <1y age class on the county level. The time of observation is expressed as calendar quarters. Frequency is expressed as the number of counties. ASFV – African swine fever virus; <1y – wild boar less than one year of age.

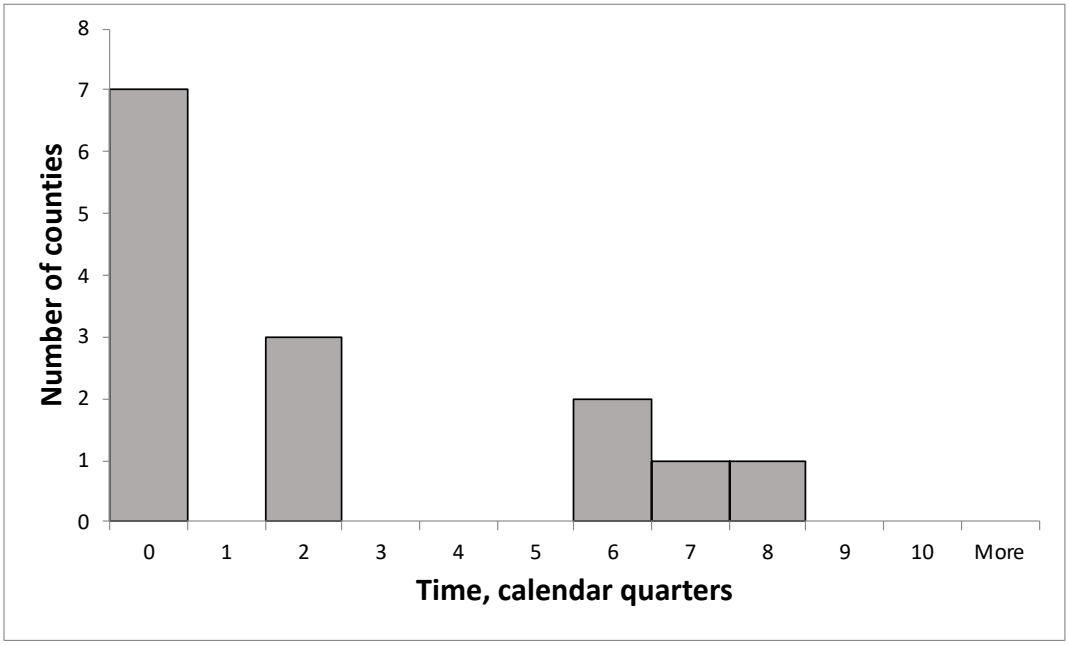

Figure 15. Frequency distribution of the time from detection of the last ASFV-positive animal to the detection of the last seropositive animal in the <1y age class on a county level. The time is expressed as calendar quarters. Frequency is expressed as the number of counties. ASFV – African swine fever virus; <1y – wild boar aged less than one year of age.

Table 7. Descriptive statistics of the duration of observation of ASFV-positive and seropositive animals, seropositive animals in all age classes and young (<1y age class) seropositive animals in each county in Estonia in 2014-2019. The time from the last detection of an ASFV-positive animal to the last young seropositive animal is also analysed. Time of observation is expressed as the number of calendar quarters

Statistic	Observation of ASFV-positive and seropositive animals	Observation of seropositive animals, all ages	Observation of ASFV-positive animals	Observation of young (<1y) seropositive animals	Time from last ASFV-positive to last young seropositive animal
Minimum calendar quarters, number of counties (n)	13 (1)	13 (1)	6 (1)	4 (1)	0 (7)
Maximum calendar quarters, number of counties (n)	21 (2)	21 (2)	18 (1)	18 (1)	8 (1)
Median	17,50	16,50	11,00	11,50	1,00
Mean	17,29	16,93	11,14	11,92	2,36
Standard deviation	2,31	2,37	2,64	3,49	2,92

Notes:

- ASFV – African swine fever virus
- <1y – young wild boar, less than one year of age
- n - number

The time period of when either ASFV-positive or seropositive were detected ranges from 13 to 21 calendar quarters (Figure 14), with the mean being 17,29 calendar quarters (Table 7). The time of detection of ASFV-positive animals (Figure 14) in most counties was 11-13 calendar quarters (9 of 14 counties), with the mean being 11,14 calendar quarters and the maximum 18 calendar quarters (one county) (Table 7). When considering the period of time when seropositive animals in any age class were detected, it was 15-18 calendar quarters in most counties (9 of 14 counties) and 16,93 calendar quarters on average, with the maximum being 21 calendar quarters (two counties). Seropositive animals in the <1y age class were detectable for a more variable time period – 10-15 calendar quarters (10 of 14 counties), and the average time period of detection was 11,92 calendar quarters, with the maximum being 18 calendar quarters (one county). The duration of the epidemic could be estimated to be 18 calendar quarters, based on the maximum time of detection of ASFV-positive animals and seropositive animals in the <1y age class (Figure 13, Table 7).

The time period of detection of seropositive animals in the <1y age class after the detection of the last ASFV-positive animals was zero calendar quarters in 7 of 14 counties (Figure 15). In these counties, the detection of the last seropositive animal in this age class occurred before the detection of the last ASFV-positive animal. The average time of detection of the last ASFV-positive animal to the detection of the last seropositive animal (Table 7) in the <1y age class was 2,36 calendar quarters, with the standard deviation being 2,92, which is higher than the mean.

4. DISCUSSION

ASF is a disease with a wide range of clinical signs. The virological and serological responses to infection also differ in individual animals. In some cases, pigs do not develop a detectable viremia or antibody response. There are also cases when pigs are apparently convalescent but experience a relapse of fever (Gallardo *et al.*, 2017; Petrov *et al.*, 2018).

There is a lack of specific and current data about some of the questions raised by this study, i.e. the prevalence, age structure and dynamics of wild boar that survive ASF. Data that is available from studies done in Africa (Allaway *et al.*, 1995) may not necessarily be relevant in the context of the current pandemic in Europe and Asia, because ASFV has adapted to a different epidemiological cycle and circulates in different reservoirs there. In contrast to Africa, most areas in Europe and Asia lack the biological vector of ASFV – ticks of *Ornithodoros* species (Chenais et *al.*, 2018; Petrov *et al.*, 2018).

In Europe, there is an apparent seasonal peak in notifications of ASF in both wild boar and domestic pigs. It is not, however, clear whether this is an actual occurrence or if it is due to a bias (EFSA, 2018).

The subject of subclinical ASFV carriers is a relevant field of study of ASF as a disease in terms of their role in ASF epidemiology. Petrov *et al.* (2018) point out the need to define and differentiate the terms “long-term persistence”, “persistent infection” and “chronic infection” – this would be important for further research and discussion.

Detection of animals that have had a previous exposure to ASFV and survived can be done by conducting serological studies. Thus, the prevalence of such survivors may be determined. Age structure and dynamics should also be determined, as this may affect ASF control strategies. It could even be argued that lack of research and current data about animals that are more likely to be affected by ASF and those more likely to survive is one of the factors that have contributed to the current epidemic still being uncontrolled (Kouokam *et al.*, 2013).

Passively derived anti-ASFV antibodies significantly alter the course of ASF in neonatal and young pigs. They have a delayed onset of clinical signs, lower viremia titers and a significantly higher, survival rate than neonates who have not received colostral anti-ASFV antibodies. This does not protect them from a subsequent infection with ASFV (Schlafer *et al.*, 1984a and b).

There is a need for studies on the subject of long-term persistence of neutralising anti-ASFV antibodies in young pigs. In the context of classical swine fever, oral vaccination of animals less than one year of age has been unsatisfactory in the past. A likely reason for this is the interference of maternal immunity (Müller *et al.*, 2005). In the context of control of ASF, this should be kept in mind, should vaccine development against ASFV be successful in the future. This also underscores the importance of determining the age profile of seropositive animals in the wild boar population. Control strategies in general need to be adjusted based on this, as not all of them will be effective in all age classes. In areas that are affected by ASF, oral vaccination of young wild boar may not be successful.

It is notable that peaks in average viroprevalence (Figures 8-10) during the ASF epidemic, are followed by a peak in average seroprevalence in the <1y age class and ≥1y age class 3-4 calendar quarters later. If there is a second peak in average viroprevalence, as in the South-east and West (Figures 8 and 10), this is followed by a second peak in the average seroprevalence in both age classes, approximately 2-3 calendar quarters later. This is also roughly reflected by Table 4, which presents average viroprevalence and seroprevalence on a yearly basis. In most cases, the highest average viroprevalence per year is followed by the highest average seroprevalence in the following year in both age classes. These findings have a rather consistent pattern, and thus may help understand and predict the dynamics of ASF spread in the wild boar population. In a prospective sense, if there is a sharp rise in viroprevalence, a rise in seroprevalence may occur, on average, half a year to a year later. If there is a subsequent peak in viroprevalence, the corresponding rise in seroprevalence on the regional level might occur sooner, especially in young wild boar (approximately half a year later).

When illustrating the dynamics of the seroprevalence during the ASF epidemic in Estonia (Figures 8-10 and 13) and evaluating the results of statistical analysis (Table 5), it becomes

apparent that the dynamics of average seroprevalence in different age classes vary in different points in time. During the first two to three years of the epidemic, the average seroprevalence in the <1y age class tends to be higher than in the ≥1y age class. The graphs (Figures 8-10) also show that the highest peaks in average seroprevalence on the regional level always occurred in the <1y age class, and this was true for all three regions in Estonia. The highest peaks in average seroprevalence in the ≥1y age class never approach those of the <1y age class. After 2017 in all three regions, there was a sharp decrease in the average seroprevalence in the <1y age class and thereafter, until the end of the study period, the seroprevalence in the ≥1y age class tended to be higher. This was shown to be statistically significant when comparing prevalence ratios (Table 5), especially comparing the average seroprevalence in the <1y age class and the >2y age class. Of the PR calculated for these age classes in 2017-2019 (South-east), 2018-2019 (North-east) and 2016-2019 (West), most were found to be statistically significant. In the beginning of the epidemic (2015-2016), when comparing the average seroprevalence of the <1y age class to the 1-2y and >2y age classes, that of the <1y age class was higher than either of the other age classes, and this was found to be statistically significant in most cases.

On the country level (Figure 13), the average viroprevalence, and the average seroprevalence in the <1y age class decreases to 0,00% starting from the 19th calendar quarter onwards. The average seroprevalence in the ≥1y age class never falls to 0,00% but shows a rise to 5,00% at the end of the study period. This is likely not an indication of new infections in this age group, but rather an accumulation of seropositive animals in older generations in later stages of the epidemic. The analysis of the dynamics of viroprevalence and seroprevalence in the first 2-3 years of the ASF epidemic likely reflects the true dynamics of ASF spread in the wild boar population. The findings described above show that new ASF infections may be more frequent in young wild boar (i.e. the <1y age class).

Similar findings were made by Nurmoja *et al.* (2017b) – they investigated the epidemiological courses of ASF in wild boar in the South and North regions of Estonia in 2014-2016, which would correspond to the beginning of the study period in the present study and the beginning of the ASF epidemic in Estonia. They found that there was a higher probability of detecting an ASFV-positive or seropositive animal (via real-time PCR and

ELISA, respectively) was higher in young wild boar (i.e. younger than one year of age) than in older age classes.

Analysis of the age structure of seropositive animals during the ASF epidemic in Estonia provides information about the of dynamics of these animals. It also helps to find patterns in these dynamics. Determining the age profile and dynamics of seropositive animals (also in relation to viroprevalence) can help identify which phase of the epidemic the wild boar population is currently undergoing.

Correlation analysis (Table 6; Figures 11 and 12) revealed that there was a statistically significant positive correlation between the number of ASFV-positive animals and the number of seropositive animals in the <1y age class and the 1-2y age class, respectively. There was also a statistically significant positive correlation between the average viroprevalence and the average seroprevalence in the <1y age class and 1-2y age class, respectively. This indicates that an increased number of young seropositive wild boar could be related to the presence of ASFV-positive animals in the wild boar population in a region. Furthermore, it could potentially indicate ongoing spread of ASFV in the wild boar population. The hypothesis outlined in the aims of the study was confirmed by univariable analysis. It should be kept in mind that the effects of other factors need to be investigated in future studies by performing multivariable analysis.

The duration of the ASFV epidemic on the county level in Estonia could be estimated to be 18 calendar quarters (approximately 54 months) at maximum, starting from the time of ASF incursion into the territory of a county. From the 19th calendar quarter onwards, the average viroprevalence and seroprevalence in the <1y age class falls to 0,00% (Figure 13).
This is approximately reflected by the frequency distributions of the time of detection of ASFV-positive and seropositive animals on the county level (Figure 14) as well as the descriptive statistics of these (Table 7). The mean time of detection of ASFV-positive and seropositive animals of all age classes was 17,29 calendar quarters. The maximum period of time of 21 calendar quarters (the mean being 16,93 calendar quarters) reflects the fact that seropositive animals in the ≥1y age class were still be found at the end study period, while seropositive animals in the <1y age class and ASFV-positive animals were not. They were found for a maximum duration of 18 calendar quarters (one county, respectively). When taking only the time period of observation of ASFV-positive animals and seropositive

animals in the <1y age class into account (Table 7), the mean period of detection was 11,14 and 11,92 calendar quarters, respectively.

There was a wide variation of how long seropositive animals in the <1y age class could be detected after the detection of the last ASFV-positive animal (Table 7). The mean period of time was 2,36 calendar quarters, but the standard deviation was 2,92, which is larger than the mean. This reflects the fact that in seven of 14 counties, ASFV-positive animals could be found for a longer time than seropositive animals in the <1y age class, and the period of time in these counties was zero calendar quarters. In one county, seropositive animals in this age class could be detected for eight calendar quarters after the detection of the last ASFV-positive animal. If this variation is not taken into account, the mean is misleading.

5. CONCLUSIONS

Review of the current literature on ASF reveals a lack of current data on the age structure and age dynamics of pigs that survive it (i.e. are seropositive). Therefore, it indicates a direction for future research. Determining the age structure and age dynamics of animals that survive ASF is an important aspect in the epidemiological characterisation of this disease, vitally important for the design and implementation of effective control and prevention strategies.

There is concern that pigs, particularly wild boar, that survive ASF may continue to carry the virus asymptomatically and continue spreading it to naïve populations. This is a concern not because there is concrete evidence of asymptomatic carriers of ASFV, but because the existence of such carriers would have a significant negative effect on efforts to control and eradicate ASF in affected areas. In the wild boar population, asymptomatic ASFV carriers would be a difficult factor to control, should it become apparent that they do perpetuate ASF spread by transmitting ASFV to naïve animals for a prolonged period of time.

Most research data indicate that animals that survive ASF eliminate the virus fully and do not spread it to naïve animals. It should be kept in mind, however, that this area still requires further research. The terms “ASFV carrier”, “chronic ASFV infection”, “persistent ASFV infection” require universally agreed-upon definitions, otherwise studies may present results that contradict each other, even when using the same ASFV isolate.

The aim of this study was to describe and analyse the dynamics of the African swine fever epidemic in the wild boar population in Estonia. Particular emphasis was placed on wild boar that survive the disease (seropositive wild boar). The age structure of seropositive wild boar in different age classes as well as the viroprevalence was determined on a calendar quarter basis on a regional level. Statistical analysis was performed to determine if the differences in average seroprevalence in young wild boar compared to the older age classes are statistically significant. The hypothesis was that the presence and increased seroprevalence in young wild boar is related to the presence of ASFV-positive animals. To

determine if this hypothesis is true or not, correlation analysis was performed, and the association of the number of ASFV-positive animals and average viroprevalence versus the number of seropositive animals and average seroprevalence in each age class was determined. In addition, the aim was to estimate the duration of the African swine fever epidemic in Estonia by determining the period of time when ASFV-positive animals and seropositive animals in each age class were detected on a county level as well as evaluating frequency distributions and descriptive statistics. For each county, the period of time of observation of young seropositive animals after the detection of the last ASFV-positive animal was determined.

Analysis of data revealed that, at the start of the ASF epidemic in Estonia, there was a higher average seroprevalence among young wild boar (i.e. less than one year of age) compared to older age classes. Towards the end of the study period, there was a higher proportion of seropositive animals in the older age classes. In most cases, these findings were statistically significant. Evaluation of descriptive statistics also revealed that, on a county level, seropositive wild boar in older age classes were detected for a longer period of time than young seropositive and ASFV-positive wild boar. On a country level, the average seroprevalence in wild boar one year of age or older actually increased slightly at the end of the study period. This is most likely not an indication of new infections in this age group but rather an accumulation of seropositive animals in the older generations. The data of the first two to three years of the ASF epidemic in Estonia likely reveal the true dynamics of ASF spread in the wild boar population, and that new ASF infections may be more frequent in young wild boar.

Correlation analysis revealed a statistically significant positive correlation of the number of ASFV-positive animals with the number of seropositive animals in the younger age classes. The average viroprevalence was also found to have a statistically significant positive correlation with the average seroprevalence in these age classes. The hypothesis that the presence and increased seroprevalence in young wild boar is related to the presence of ASFV-positive animals was confirmed with univariable analysis. Further research needs to be done in future studies, the influence of other factors needs to be analysed by conducting multivariable analysis.

The findings described above show the practical value of performing serological studies in the wild boar population, determining the age profile of seropositive animals in different points in time, and determining the association of the number of ASFV-positive animals and seropositive animals in different age classes. If there is a marked increase in the number and prevalence of seropositive young wild boar, this could potentially serve as an indicator of new infections in the wild boar population and ongoing spread of ASFV. The age profile of seropositive animals may help indicate which phase of the ASF epidemic the wild boar population is currently undergoing.

Retrospectively determining the duration of observation of ASFV-positive animals and seropositive animals in different age classes in each county could potentially be a valuable tool for determining the duration of the ASF epidemic on the country level. The most likely indicators of the duration of the ASF epidemic in Estonia were the duration of observation of ASFV-positive and seropositive young wild boar. Seropositive wild boar in the older age classes were still found at the end of the study period, while seropositive young wild boar and ASFV-positive wild boar were not. Based on these findings, it could be suggested that the duration of the ASF epidemic in Estonia on the county level was 18 calendar quarters (approximately 54 months) at maximum. From the 18th calendar quarter onwards, no ASFV-positive or young seropositive wild boar were detected in any county.

This study provides insight into the dynamics of ASF in the wild boar population in the context of the ongoing epizootic. It provides information on aspects of the epidemic where there was a lack of current data and helps indicate directions of future research. It can also serve as a potential framework for analysing the dynamics of ASF in the wild boar populations in other countries. This is crucial for devising control measures to halt the spread of ASF, protect unaffected areas, and eradicate this disease.

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